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Health Assessment Document for Dichloromethane (Methylene Chloride)

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Health Assessment Document for Dichloromethane (Methylene Chloride)

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16. ABSTRACT

Dichloromethane (methylene chloride) is known to be metabolized to carbon monoxide in man and animals, primarily by the liver. Because the oxygen content of blood is decreased, depriving the brain and heart of the oxygen they require, serious permanent damage may result. Carboxyhemoglobin is formed from the interaction of carbon monoxide and hemoglobin. Persons exposed to levels of dichloromethane that do not exceed the industrial standard of 500 ppm may have blood carboxyhemoglobin levels that exceed those allowable from direct exposure to carbon monoxide.

The adverse health effects associated with dichloromethane exposure are primarily neurological and cardiovascular. There is evidence that exposure can result in hemolytic anemia, especially among those individuals with erythrocytes deficient in glucose-6-phosphate dehydrogenase. The observed cardiotoxic properties include cardiodepression and cardiosensitization. Nonfatal exposures have caused EKG changes similar to those caused by carbon monoxide.

The existing data base is inadequate for assessing the carcinogenicity of dichloromethane. On the basis of limited data available, teratogenic risk after exposure in minimal. Dichloromethane, however, has been demonstrated to cross the placenta and fetal accumulation has been observed. Dichloromethane has produced positive responses in bacterial and yeast mutagenicity systems but the purity of the test material is unknown.

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NOTICE

This document is a preliminary draft. It has not been formally released by EPA and should not at this stage be construed to represent Agency policy. It is being circulated for comment on its technical accuracy and policy implications.

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PREFACE

The Office of Health and Environmental Assessment, in consultation with an Agency work group, has prepared this health assessment to serve as a "source document" for Agency-wide use. Originally the health assessment was developed for use by the Office of Air Quality Planning and Standards, however, at the request of the Agency Work Group on Solvents, the assessment scope was expanded to address multimedia aspects. This assessment will help insure consistency in the Agency's consideration of the relevant scientific health data associated with methylene chloride.

In the development of the assessment document, the scientific literature has been inventoried, key studies have been evaluated and summary/conclusions have been prepared so that the chemical's toxicity and related characteristics are qualitatively identified. Observed effect levels and dose-reponse relationships are discussed, where appropriate, so that the nature of the adverse health responses are placed in perspective with observed environmental levels.

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1. SUMMARY AND CONCLUSIONS

Dichloromethane (methylene chloride, DCM) is a solvent widely used for a variety of purposes. United States annual production of dichloromethane has averaged around 227,000 metric tons (about 500 million pounds) in recent years; an increase in production is expected. It is estimated that approximately 85 percent of the dichloromethane consumed in the United States is lost directly to the environment through dispersive use, largely by evaporation to the atmosphere. Natural sources have been proposed for dichloromethane, but none is presently believed to contribute significantly to ambient concentrations. Ambient air and water measurements, although rather scarce, indicate that dichloromethane is found in a variety of urban and non-urban areas of the United States and in other regions of the world. The background atmospheric concentration is about 35 parts per trillion (ppt). Concentrations of dichloromethane in urban areas do not seem to be significantly higher than in non-urban areas; the maximum concentration measured in urban areas is 12 parts per billion (ppb) detected in Los Angeles, California, 1979. Some extremely high concentrations of DCM from nearby sources have been measured in indoor air. Dichloromethane is not expected to accumulate in the atmosphere. Estimates of half-life vary from 20 days to one year. Hydroxyl-free radical attack is probably sufficiently rapid to prevent most, if not all, dichloromethane from reaching the stratosphere. Rainout is not considered to cause a significant reduction in atmospheric dichloromethane.

Dichloromethane has been detected in both natural and municipal waters in various geographical areas of the United States. It has not been measured in seawater. Concentrations of dichloromethane have been measured in surface water and finished drinking water in the low ppb range. Dichloromethane does not appear to be formed to any large extent during the chlorination process. Its short evaporation half-life from moving water probably allows most of the compound dissolved in water to be eventually transported into the atmosphere. It is also readily degraded by bacteria in concentrations up to 400 parts per million (ppm). The extent to which dichloromethane enters groundwater from surface waters is unknown. Some dichloromethane is deposited in landfills; where leaching is possible the compound may enter groundwater systems because it adsorbs very little to clay, limestone, and/or peat moss, so retention in the soil is unlikely. There is no evidence of its significant bioaccumulation in the food chain. Little information on the ecological consequences of dichloromethane in the environment is available.

The pharmacokinetics and metabolism of dichloromethane have not been extensively studied in humans, although a broad outline of its absorption, distribution, metabolism, and elimination has been established. As with other solvents of this class, inhalation of dichloromethane in air followed by lung absorption is the most rapid route of entrance into the body. Dichloromethane is also well absorbed into the body after oral ingestion. Absorption through the intact skin occurs to some extent, but is relatively a much slower process. Dichloromethane is appreciably more water-soluble and less lipid-soluble than its congeners, carbon tetrachloride and chloroform. Because of its solubility in water and lipids, dichloromethane probably distributes throughout all body fluids and tissues. It readily crosses the blood-brain barrier, as evidenced by its narcotic effect. It also crosses the placenta and distributes into the developing fetus.

Its long half-time of elimination from adipose tissue (6 to 6½ hr), together with reports that it remains in such tissue 24 hours after both single and chronic exposures, indicate that dichloromethane may very slowly accumulate in body fat with long daily exposures to high air concentrations. The risk of accumulation might be expected to be greater for obese persons than for lean persons.

Following oral ingestion, the absorption of dichloromethane is virtually complete; with ambient air exposure, the amount absorbed increases in direct proportion to its concentration in inspired air, the duration of exposure, and physical activity. Absorbed dichloromethane is eliminated primarily by pulmonary excretion of the unaltered parent compound (about 85 percent). About two percent is excreted unchanged in the urine, while a small amount is eliminated by other routes. Ten percent or less is metabolized before elimination from the body. At very low concentrations essentially all of the compound absorbed into the body may be metabolized. Dichloromethane is known to be metabolized to carbon monoxide (CO) in man as well as in animals, primarily by the liver. This metabolism of halogenated hydrocarbons to CO is apparently unique to the dihalomethanes. Carboxyhemoglobin (COHb) is formed from the interaction of CO and hemoglobin; CO dissociates at the lung and is eliminated. However, the oxygen content of the blood is decreased, depriving the brain and heart of the oxygen they require. Serious permanent damage may result.

The endogenous production of CO, and then COHb, from dichloromethane metabolism is additive to COHb formed from exogenous CO. For this reason, persons exposed to levels of dichloromethane that do not exceed the industrial standard of 500 ppm (1737 mg/m^3) may have blood COHb levels that exceed those allowable from direct CO exposure. Dichloromethane exposure may thus result in toxicities associated with the solvent as well as with CO. The results of animal experimentation by several investigators indicate that carbon dioxide, formaldehyde, and formic acid are additional metabolites of dichloromethane.

At least two pathways exist in rat liver for the metabolism of dichloromethane. Together, the microsome oxidative dehalogenation and cytosol glutathione transferase dehalogenation systems account for the CO and CO_2 generated from the metabolism of dichloromethane. The microsomal system, but not the cytosol system, is inducible by phenobarbital and other microsomal inducers, and by dichloromethane itself.

The adverse health effects associated with dichloromethane exposure are primarily neurological and cardiovascular. The increased blood levels of carboxyhemoglobin that are a consequence of metabolic transformation of dichloromethane to carbon monoxide may result in permanent damage to the brain and heart. In addition, the teratogenic, mutagenic, and carcinogenic potentials of this chemical are possible causes for concern. There is evidence that exposure to dichloromethane can result in hemolytic anemia, especially in certain populations, e.g., persons having erythrocytes deficient in glucose-6-phosphate dehydrogenase. Liver and kidney morphological damage are probably somewhat less likely to result from dichloromethane exposure than from exposure to similar solvents such as chloroform and carbon tetrachloride. Contact of the eyes, skin, and respiratory mucosa with dichloromethane will cause local irritation.

The observed cardiotoxic properties of dichloromethane include cardiodepression and cardiosensitization. Several human case studies have reported fatalities resulting from, or closely associated with, exposure to dichloromethane, in which myocardial infarction was diagnosed. Nonfatal exposures have caused electrocardiographic (EKG) changes similar to CO-induced EKG changes. The relative contributions of dichloromethane and its metabolite, CO, to these effects are unclear. The histories of many exposed individuals having symptoms of cardiotoxicity suggest the existence of underlying cardiovascular disease. Cardiotoxic effects may therefore be significant to this subpopulation.

Animal studies have indicated that the primary cardiovascular effect of dichloromethane is decreased myocardial contractility. Dichloromethane was also capable of sensitizing the heart to epinephrine-induced arrhythmias. While the levels of epinephrine used in these studies were somewhat higher than those normally occurring during a stress reaction, they may suggest an increased susceptibility of certain individuals to the effects of dichloromethane.

Hepatotoxicity has not been reported in any human case report, even following fatal exposures. Animal studies have reported only minimal hepatic changes, even at doses ranging from the LD_{50} to "near lethal" doses. Some species differences occurred (dogs were more susceptible than mice) but the liver changes were nonetheless generally minor.

The only evidence of human nephrotoxicity resulting from dichloromethane exposure was the finding of congested kidneys following a fatal exposure. Animal studies concur, although the number of studies describing renal changes was small. In contrast to hepatotoxicity, mice were more susceptible to nephrotoxicity than dogs, but again, the changes reported were minimal.

There are reports of fatal and nonfatal acute human effects occurring after exposure to dichloromethane. Inhalation exposures or concurrent inhalation and dermal exposures were the most common. Although the concentration of dichloromethane has usually not been reported, the circumstances under which the human exposures occurred suggest that the concentrations have ranged from very high levels in industrial accidents to more moderate levels associated with home use of consumer products, specifically removers of paint and varnish.

Animal studies generally support and confirm the toxic effects noted in such cases.

Central nervous system (CNS) effects are related to the anesthetic properties of dichloromethane. The onset of these effects is generally rapid and they are temporary, normally subsiding within hours after the cessation of exposure. In cases of acute human exposure, CNS effects have included death, unconsciousness, labored breathing, headache, lassitude, and nausea. Behavioral and neurological alterations resulting from damage to central and peripheral nervous systems have been reported following acute exposure. The onset of symptoms ranged from immediate to several months after exposure. Some of these effects were prolonged, persisting for at least 20 months after the exposure. Progressive bilateral temporal lobe degeneration, as a consequence of dichloromethane exposure, has been associated with memory loss, general mental deterioration, headache, and dysarthria. Dichloromethane-induced toxic encephalosis, resulting in visual and auditory illusions and hallucinations, has been reported. Dichloromethane has been reported to decrease peripheral nerve conduction and to induce abnormal rapid eye movement (REM) sleep in rats.

There is less agreement on the human toxicology of dichloromethane from low-level, long-term exposures. Experimental animal studies and evidence in humans provide limited information on the correlation between chronic exposure to dichloromethane and subsequent toxic effects. Difficulties in delineating the toxic effects of dichloromethane are further compounded by chemical impurities. Exposure to levels of dichloromethane close to its threshold limit value (TLV) have evoked subjective responses that suggest CNS involvement or are of psychosomatic origin. Studies of behavioral effects, particularly as they affect manual and cognitive performance, have reported impairment at TLV levels. Decrements in eye-hand coordination and task-related response time have been associated with carboxyhemoglobin levels of 3 to 5 percent.

Many studies have shown that COHb levels of 2.5 percent or greater can adversely affect individuals with angina pectoris or cardiovascular disease. Thus exposures to dichloromethane that increased the body burden of COHb can further stress individuals who are already compromised by decreased oxygen transport capabilities or cardiovascular disease. Chronic or subchronic exposure to dichloromethane at concentrations from 50 to 200 ppm were found to increase COHb levels to 2.9 to 4.5 percent in both workers and non-smoking experimental volunteers.

An important and most controversial aspect of dichloromethane is its teratogenic, mutagenic and carcinogenic potential. On the basis of the limited data available, the teratogenic risk after exposure to dichloromethane is minimal; however, there is a preliminary report suggesting the possibility of delayed behavioral effects in offspring following exposure. Dichloromethane has been demonstrated to cross the placenta, and fetal accumulation of dichloromethane has also been demonstrated. Higher concentrations of dichloromethane have been found in human cord blood and fetal tissue than in maternal blood.

There are positive responses for bacterial and yeast mutagenicity, but information on the purity of the test compounds is not yet available. Dichloromethane also showed an ability to transform cells using the rat embryo cell line F1706. This was not confirmed when a reportedly purer grade of dichloromethane was tested.

The existing data base is inadequate for assessing the carcinogenicity of dichloromethane. There is a marginally positive pulmonary adenoma response in strain A mice. Two negative animal inhalation studies were inadequate because they were not carried out for a full lifetime. Two long-term animal bioassay studies are currently in progress at the National Toxicology Program (NTP)--a gavage test nearing completion and an inhalation test recently started. The animals in the NTP gavage study were sacrificed in December, 1980. A chronic DCM inhalation study sponsored by industry has been conducted in rats and hamsters. The rat study showed a small but statistically significant increase in incidence of benign mammary tumors in female rats at all doses, and in male rats at the highest dose. There was also a significant increase in sarcomas, probably of salivary gland origin, in male rats. The response pattern of the salivary gland tumors is unusual: they appeared only in males and consisted only of sarcomas. In hamsters, there was an increased incidence of lymphosarcomas, in females only, which was not statistically significant after correction for survival.

Only one occupational epidemiological study of dichloromethane has been reported, and it showed no increased incidence of neoplasms that could be related to dichloromethane at any sites. However, the study was insensitive to latent tumor development due to the short duration of the follow-up.

Mortality studies of a worker population in which individuals had been exposed to dichloromethane failed to reveal any signfiicant excess deaths by major diagnostic or malignancy groupings when 751 employees exposed to the chemical were compared with industrial workers not exposed to dichloromethane and with New York State male populations.

A final assessment of the carcinogenicity of dichloromethane by the EPA Carcinogen Assessment Group (CAG) will be deferred until information on the purity of the material used in the positive mutagenicity tests is obtained and until the results of the NTP gavage bioassay are evaluated.

2. INTRODUCTION

Dichloromethane is a high production industrial chemical. In view of an apparently general belief that this compound is in many respects less toxic than several of the other commonly used solvents, annual production may be expected to increase. The 16,500 metric tons (36 million pounds) of dichloromethane produced in the United States in 1951 doubled to 33,500 metric tons (74 million pounds) in 1955 and exceeded 227,000 metric tons (500 million pounds) in 1976. Yearly production was only 217,000 metric tons (478 million pounds) in 1977 but jumped to 297,000 metric tons (654 million pounds) in 1979, and was estimated to be 244,000 metric tons (537 million pounds) in 1980. Any leveling trend in the manufacture of this chemical is not likely to continue, however, and overall production should increase.

Dichloromethane is used in large quantities for a variety of purposes. Approximately 30 percent is used for paint removal. Dichloromethane is preferred for this purpose over competitive solvents because of its nonreactivity with aluminum. Twenty percent is used as a degreasing agent and another 20 percent is used as a propellant for aerosol sprays. Since dichloromethane is replacing many chlorofluorocarbons, the aerosol sector is expected to become its largest market. Principal categories of dichloromethane usage during 1977 and 1978 are shown in Table 2-1.

Approximately 85 percent of the dichloromethane produced is emitted into the environment from product manufacture and use. Most of the losses of this chemical are through dispersion into the atmosphere, although the potential exists for concentrations up to 1,500 ppm to occur in water effluents from its use as an extraction solvent. The use of dichloromethane is likely to result in measurable human exposure. In the past, the primary health concern was for

TABLE 2-1. CONSUMPTION OF DICHLOROMETHANE

Use	Metric tons (10^3) (1980)	Percent Total (1977)	Percent Total (1978)
Paint remover	73	30	29
Metal degreasing agent	49	20	18
Aerosol propellant	46	19	21
Blowing agent for foams (urethane)	20	8	9
Exports	44	18	15
Other	12	5	8

SRI International. Chemical Economics Handbook, 1980.

exposed industrial workers. Since dichloromethane is an ingredient in many consumer products, however, millions of people are likely to be exposed to the chemical at home. The concern for health effects has broadened with the realization that most of each year's production is released into the environment and contaminates the atmosphere, raw water sources, drinking water, soil, and possibly the food chain. The large population exposed to dichloromethane includes various susceptible subgroups that may be particularly sensitive to its adverse health effects, i.e., pregnant women, infants and children, the elderly, and those with compromised cardiovascular systems. In addition, the problem of possible additive or synergistic effects is also troublesome when there is simultaneous exposure to other halomethanes or to certain medications with which dichloromethane may interact adversely.

This document is intended to provide an evaluation of the current health hazards of dichloromethane that are associated with exposure levels encountered in the workplace as well as the lower levels expected in the environment and, therefore, encountered by the population at large. Both the older and the more current scientific literature has been reviewed and evaluated. The abbreviation DCM has been used for dichloromethane.

To give perspective to the evaluation of the health hazards from exposure to DCM and a foundation for better comprehension of the data presented in subsequent chapters, Chapter 3 provides basic background information about the physical and chemical properties of DCM; its production, sources and emissions; its atmospheric transport, transformation and fate; ambient air, water, and soil concentrations; analytical methods for detection and quantification; and ecological effects.

The physical and chemical properties of DCM are given in Table 2-2. As can be noted from this table, DCM is highly volatile, with a vapor pressure of

TABLE 2-2. SELECTED PROPERTIES OF DICHLOROMETHANE

Molecular formula	CH_2Cl_2
Formula weight	84.94
Boiling point (760 mg Hg)	40°C (760 mm Hg)
Melting point	-95 to -97°C
Vapor density	2.93 (air = 1)
Density of saturated vapor	2.06 (air = 1)
Density	1.326 g/ml (20°C)
Solubility	2.0 g/100 ml water at 20°C; soluble in ethanol, ethyl ether, acetone, and carbon disulfide
Explosive limits in oxygen	15.5-67% by volume
Flash point	None
Autoignition temperature	624 - 662°C
Relative evaporation rate	14 (water = 1) 71 (ether = 100)
Vapor pressure	Temp F / Temp C / mm HG 50 / 10 / 230 68 / 20 / 349 77 / 25 / 436 86 / 30 / 511 95 / 35 / 600
Conversion factors (25°C; 760 mm Hg)	1 mg/liter = 1 g/cu m = 288 ppm 1 ppm = 3.474 mg/cu m = 3.474 μg/liter
Concentration in saturated air	550,000 ppm (25°C)

Hardie, 1969.

Weast, 1969.

American National Standards Institute Inc., 1970.

Christensen and Luginbyhl, 1974.

350 torr at room temperature. Since the ambient air concentration for compounds of this nature is often expressed in parts per million (ppm), the appropriate conversion factors for the metric system (mg/l and g/m^3) are also included in this table.

Despite large emissions, DCM has been measured only a few times in the atmosphere because of its relatively short atmospheric life of less than one year and because of the lack of sensitivity of electron capture detectors for a compound having only two chlorine atoms per molecule. The average background atmospheric concentration of DCM is approximately 35 ppt with little variation between continental and marine air. Urban concentrations vary from low background levels of 20 ppt to a maximum concentration of 12 ppb. Relatively very little DCM reaches the stratosphere. The chemical has been found in concentrations in the low ppb range in raw surface water and subsurface water, as well as in finished drinking water, where it is formed during the chlorination process of water treatment. The short evaporation half-life (21 min) of DCM from moving water probably allows most of the compound dissolved in water to be eventually transported into the atmosphere. Concentrations of up to 400 ppm DCM are also readily degraded by bacteria. Formation of DCM from natural sources occurs but is not believed to contribute significantly to global concentrations. DCM is deposited in landfills where some release to the atmosphere through evaporation is likely. Where leaching is possible, DCM may enter groundwater systems as retention in the soil is unlikely. Elevated levels of DCM have been measured in the indoor environment. The common sources of exposure to DCM and their potential to affect human health are reviewed in Chapter 3.

DCM is readily absorbed into the body, through the lungs by inhalation of its vapors and through the gastrointestinal tract after oral ingestion in water and food. Absorption through the skin from direct contact with liquid DCM appears to be a slower process. At concentration levels allowable in the workplace, more than 85 percent of DCM is excreted unchanged via the lungs. A small amount of DCM is eliminated unchanged in urine and by other routes; 10 percent or less is metabolized prior to elimination. However, essentially most of the compound absorbed may be metabolized at very low concentrations. Biotransformation occurs primarily in the liver, and to a lesser extent in the lungs and kidneys. DCM distributes to all body tissues. It is known to be metabolized in animals and in man to carbon monoxide (CO), which elevates the carboxyhemoglobin content of blood. It has a relatively long half-time of elimination from adipose tissue. The distribution, storage, and metabolism of DCM to CO, formaldehyde, and formic acid help to explain its human toxicity.

Toxicity may be enhanced by prior or concurrent exposure to certain clinical drugs, exogenous CO, and some xenobiotics. These aspects of the disposition and fate of DCM, as concluded from animal studies as well as from methods of determining and quantifying exposure intensity and their limitations, are discussed in Chapter 4.

At high concentration levels (>1000-2000 ppm), DCM is a central nervous system depressant and an anesthetic and can cause injury to the CNS, liver, and kidneys. It is irritating to the eyes, skin, and respiratory mucosa. Because the affinity of its metabolite, CO, for hemoglobin is more than two hundred times greater than that of oxygen (O_2), the CO interacts preferentially with hemoglobin to form carboxyhemoglobin, which deprives the brain and heart of the oxygen they require, possibly resulting in serious permanent damage.

At lower level exposure, about 20 to 500 ppm DCM has behavioral and psychological effects that alter manual dexterity and mental performance. The adverse effects of low-level, chronic exposure to DCM have not been as extensively studied in either man or animals. Studies of the adverse health affects of DCM at different levels of exposure are reviewed and evaluated in Chapter 5.

The teratogenic, mutagenic, and carcinogenic potential of DCM is a controversial major issue. Although DCM crosses the placenta, the evidence that it is teratogenic is minimal, and the studies are few at the present time. The possibility of delayed behavioral effects exists, but the studies are too preliminary to be definitive. Studies relating to teratogenicity, fetal toxicity and reproductive effects are reviewed and evaluated in Chapter 5.

The EPA Carcinogen Assessment Group (CAG) has deferred a final assessment of the cancer-causing activity of DCM until information on the purity of the material used in the positive mutagenicity tests on this chemical is obtained, and until the results of the 2-year National Toxicology Program (NTP) chronic gavage bioassay are obtained. This additional information may also help to clarify the rather unusual results of the Dow Chemical Company inhalation studies. Chapter 5 examines the evidence from the currently available mutagenicity studies, animal carcinogenicity studies, and epidemiological data. A more in-depth review of this information is presented in the CAG-REAG (Reproductive Effects Assessment Group) report which is included as an appendix to this health assessment document.

DCM may be present in the water we drink, the air we breathe, and the food we eat. It is produced in large quantities, most of which is released into the environment largely through evaporative loss from dispersive use. Widespread human exposure, including special susceptible populations, may occur as a result of its use in the workplace, its occurrence in consumer products, and its loss into the environment.

3. DICHLOROMETHANE: BACKGROUND INFORMATION

3.1 PHYSICAL AND CHEMICAL PROPERTIES

Dichloromethane (methylene chloride, DCM, CH_2Cl_2) is one member of a family of saturated aliphatic halogenated compounds. Other common names or synonyms for DCM include methane dichloride, methlene bichloride, methlene dichloride, Solaesthin, Aerothene MM, chlorure de methylene, and metlenu chlorek (see Table 3-1). The Chemical Abstracts Service Registry Number for DCM is 000075092. Dichloromethane is a colorless, nonflammable, volatile liquid that is completely miscible with a variety of other solvents (Anthony, 1979). Its important physical properties are shown in Table 3-2. As can be noted from this table, DCM is highly volatile with a vapor pressure of 350 torr at room temperature. Hence, the most common mode of entry into the body is by inhalation. The ambient air concentration for compounds of this nature is often expressed in parts per million (ppm). At standard temperature and pressure, 1 part per million is equivalent to 3.474 mg m^{-3}.

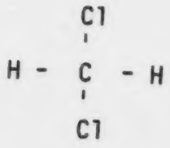
In the absence of moisture at ordinary temperatures, DCM is relatively stable when compared with its congeners, chloroform and carbon tetrachloride. In dry air, DCM decomposes at temperatures exceeding 120°C (Anthony, 1979). At elevated temperatures (300° to 450°C), it tends to carbonize when its vapor contacts steel and metal chlorides. Moisture initates hydrolysis of DCM, predominantly to hydrogen chloride (HCl) with trace amounts of phosgene (Anthony, 1979). It has been reported that even trace amounts of phosgene detract from the paint stripping qualities of DCM (De Forest, 1979). To retard production of phosgene via hydrolysis, inhibitors are generally added to commercial preparations of DCM (De Forest, 1979). Protection against hydrolysis also is attained by addition of phenolic compounds (Anthony, 1979).

TABLE 3-1. SYNONYMS AND IDENTIFIERS FOR DICHLOROMETHANE

Chemical Abstracts Service Registry Number 000075092

Chemical Formula CH_2Cl_2

Structural Formula



$$H - \overset{\displaystyle Cl}{\underset{\displaystyle Cl}{C}} - H$$

Dichloromethane
Methylene Dichloride
Methylene Bichloride
Methylene Chloride
Solaesthin
Aerothene MM
Chlorure de methylene
Metlenu Chlorek

TABLE 3-2. PHYSICAL PROPERTIES OF DICHLOROMETHANE (ANTHONY, 1979)

Molecular weight	84.94
Vapor specific gravity	2.93 grams liter^{-1} (air = 1)
Boiling point	40°C
Vapor pressure	511 mm Hg @ 30°C, 350 torr
Solubility in water[a]	13,200 (@ 20°C) to 22,700 ppm
Log octane/water partition[b] coefficient	1.25

[a] Large discrepancies in the solubility of DCM have been reported. Dilling (1977) reported values obtained from the literature ranging from 6,270 ppm (@ 20°C) to 22,700 ppm (Glew and Moelwyn-Hughes, 1953).

[b] De Forest, 1979.

Although anhydrous DCM is noncorrosive to common metals, the HCl produced from hydrolysis of DCM initiates corrosive action with aluminum and iron (Anthony, 1979). The addition of epoxides to consume the HCl affords protection (Anthony, 1979). To minimize the decomposition of DCM, storage containers should be galvanized or lined with a phenolic coating (Anthony, 1979). Commercial grades of DCM contain a variety of stabilizers to minimize decomposition (McKetta and Cunningham, 1979). Cyclohexane, thymol, hydroquinone, p-cresol, and low-boiling amines have been used (De Forest, 1979). Aerosol preparations containing DCM often use propylene oxide as a stabilizer. For preparations designed to be used in degreasing applications, special inhibitor mixtures are used. One such mixture includes propylene oxide, butylene oxide, cyclohexane, and N-methyl morpholine (De Forest, 1979).

The experimentally-determined average evaporative half-life of DCM from water is in the range of 18 to 25 minutes (Dilling, 1977). In three separate experiments, Dilling used solutions of an average depth of 6.5 cm containing approximately 1 ppm DCM. The solutions were stirred at 200 rpm in a 250 ml beaker. These experimentally-determined half-lives agreed with the value (20.7 minutes) obtained by the following formula

$$t_{\frac{1}{2}} = \frac{0.6391d}{K_1}$$

where d is the solution depth and K_1 is the liquid exchange constant (cm/min). The formula is an adaptation of the common equation for the half-life of a substance undergoing a first order reaction.

3.2 ENVIRONMENTAL FATE AND TRANSPORT

Dichloromethane is principally used as an aerosol propellant, degreasing solvent, and thinner in paints and lacquers. Because of its volatility and dispersive use pattern, much of the DCM produced worldwide is emitted into the atmosphere. The emissions are almost entirely due to anthropogenic sources. Formation of DCM from natural soruces occurs but is not believed to contribute significantly to global concentration (National Academy of Sciences, 1978).

3.2.1 Production

Dichloromethane is produced commercially in the United States predominantly via the following reaction (De Forest, 1979; Anthony, 1979):

$$HCl + CH_3OH \xrightarrow[130^\circ \rightarrow 180^\circ C]{FeCl_3 \text{ or } ZnCl} CH_3Cl + H_2O$$

$$CH_3Cl + Cl_2 \xrightarrow{\hspace{3cm}} CH_2Cl_2 + HCl$$

In this vapor phase reaction sequence, yields of 95 percent are generally experienced. Dimethyl ether is the secondary byproduct of the hydrochlorination.

A less commonly used method is direct reaction of methane with chlorine at 485° to 510°C (Anthony, 1979). Methyl chloride, chloroform, carbon tetrachloride, and HCl are coproducts. The coproducts represent a disadvantage of this method. However, both reactions are used in the chemical manufacturing industry so that the HCl can be recycled (Anthony, 1979).

In the liquid phase, DCM can be produced by refluxing and distilling a mixture containing methanol, HCl, and zinc chloride at 100° to 150°C. It is not widely used (Anthony, 1979).

According to one source, production in the United States is carried out by five major companies at seven sites (Table 3-3). However, the U.S. EPA TSCA Public Inventory showed that in 1977 there were six manufacturers and 13 importers (U.S. EPA, 1980b).

According to statistics gathered by the U.S. International Trade Commission, the United States annual production of 36 million pounds (16,500 metric tons) in 1951 doubled to 74 million pounds (33,500 metric tons) in 1955, was 471 million

TABLE 3-3. PRODUCERS OF DICHLOROMETHANE

Company	Annual Capacity (thousands of metric tons annually) as of January 1, 1979
Allied Chemical, Moundsville, WV	23
Diamond Shamrock, Belle, WV	50
Dow Chemical, Freeport, TX Pittsburgh, PA	92
Plaquemine, LA	88
Stauffer Chemical, Louisville, KY Le Moyne, AL	28
Vulcan Materials, Geismar, LA	37
Wichita, KA	60

Source: Anon. 1979 Methylene Chloride in: Chemical Marketing Reporter. August 6, 1979, p. 9.

pounds (214,000 metric tons) in 1972, exceeded 500 million pounds in 1976 (227,000 metric tons), was 478 million pounds (217,000 metric tons) in 1977, was 634 million pounds (297,000 metric tons) in 1979, and was estimated to be 537 million pounds (244,000 metric tons) in 1980. The leveling trend in annual production is not expected to continue because DCM is believed to be less toxic than most of the other commonly used solvents. In 1976, 42 million pounds (19,000 metric tons) were imported, while exports totalled 97 million pounds (44,000 metric tons) in 1975 (USITC, 1980).

3.2.2 Use

Dichloromethane is used for a variety of purposes: as a paint remover, a urethane foam-blowing agent, a vapor degreasing and dip solvent for metal cleaning, a solvent for aerosol products, a solvent in the pharmaceutical industry, a solvent in the manufacture of polycarbonates by polymerization, and as an extractant for caffeine, spices, and hops. It is used in the manufacture of plastics, textiles, photographic film, and photoresistant coatings, as a solvent carrier in the manufacture of herbicides and insecticides, and in rapid drying paints and adhesives, carbon removers and brush cleaners. Other minor applications include use as a low pressure refrigerant, as a low-temperature heat transfer medium and as an air-conditioning coolant (Ahlstrom and Steele, 1979). Distribution of DCM in its major uses is shown in Table 2-1. The fastest growing segment of the DCM market is the aerosol sector due to the substitution of DCM for chlorofluorocarbons as a solvent, vapor pressure depressant, and flame retardant. Consumption by the aerosol industry is expected to grow by as much as 15 percent annually over the next several years and to become the largest market for dichloromethane (Ahlstrom and Steele, 1979; Lowenheim and Moran, 1975).

Dichloromethane is expected to retain popularity as a paint remover. Although it competes with trichloroethylene and perchloroethylene as a solvent, it is perferred as a paint remover because of its nonreactivity with aluminum (Lowenheim and Moran, 1975).

3.2.3 Emissions

Emissions from dispersive uses are the major source of DCM in the environment. Of the total amount of DCM produced in the United States, approximately 85 percent is estimated to be lost into the environment through sewage treatment plants and surface waters, deposited on land or lost to the atmosphere. About 3/4 by weight of all annual halomethane emissions are thought to originate from dispersive uses of DCM. The actual losses during production, transport, and storage are not well documented, but it appears that such losses represent only a very small percent of the DCM entering the environment from product manufacture and use. Most of the losses in production transportation and storage are the fugitive type, that is, transient releases due to leaky pump seals, valves, and joints. The dispersive uses of DCM are varied and widespread and are distributed geographically approximately with the industrialized population in the United States. Although most of the losses are to the atmosphere, DCM is relatively soluble and, when it is used as an extraction solvent, the potential exists for concentrations up to 1,500 ppm to occur in water effluents. The most effective means of removal of DCM from water is air stripping, which transfers the chemical from water to the atmosphere (NAS, 1978).

3.2.4 Persistence of DCM

Reaction with hydroxyl radicals (OH) is the principal process by which many organic chemicals, including DCM, are scavenged from the troposphere (Crutzen and Fishman, 1977; Singh, 1977; Altshuller, 1980). These radicals are produced upon irradiation of ozone (O_3). The resultant singlet oxygen atoms [O'(D)] then react with water vapor. The tropospheric lifetime of a compound is related to the OH concentration by the expression:

$$\tau \text{ lifetime} = \frac{1}{k\,[OH]}$$

where k is the rate constant of reaction.

Recent findings (Crutzen and Fishman, 1977; Singh, 1977) imply lower tropospheric concentrations of OH than previously believed. Therefore, DCM may have a longer lifetime than commonly thought. The modelling approaches of Crutzen and Fishman (1977) and Singh (1977) indicate that range of the average OH concentration as between 2×10^5 and 6×10^5 molecules cm^{-3}. Using an average concentration of 3×10^5 molecules cm^{-3}, Altshuller (1980) has calculated a tropospheric lifetime for DCM of 1.4 years. The rate constant expression of Davis et al. (1976) and a tropospheric temperature of 265°K were used.

Singh et al. (1979) computed a 1-year lifetime for DCM using the rate data reported by NASA (1977) and NBS (1978) and a temperature of 265°K. An average OH concentration of 4×10^5 molecules cm^{-3} was employed in the computation.

Cox et al. (1976) on the other hand, calculated a 0.3 year lifetime in photokinetic studies in which DCM competed with nitrous acid as the target of OH attack. The lifetime was derived from a rate constant of 10.4×10^{-14} cm^3 $molecule^{-1}$ sec^{-1} at 298°K. An average OH concentration of 1×10^6 molecules cm^3 was assumed. Use of 4×10^5 molecules cm^3 for the OH concentration would have resulted in a calculated lifetime of 0.76 year, a value more in agreement with those of Singh (1977) and Altshuller (1980). Davis et al. (1976) calculated a lifetime of 0.39 years from a rate constant of 8.7×10^{14} at 265°K and an average OH concentration of 9×10^5 molecules cm^3.

Determination of the rate constant for the reaction of OH with DCM has been the focus of various investigations (Davis et al., 1976; Cox et al., 1976;

Howard and Evenson, 1976; Perry et al., 1976). The values are in general agreement (Table 3-4) with the exception of Butler et al. (1978).

3.2.5 Products of DCM

3.2.5.1 Atmospheric Simulation Studies--Dilling et al. (1976) observed that DCM was not very reactive in a chamber atmosphere containing nitric oxide (NO) or nitrogen dioxide (NO_2). Ozone-air mixtures containing 10 ppm DCM and 5 ppm NO or 16.8 ppm NO_2 were exposed to ultraviolet radiation (UV) at an intensity about 2.6 times that of natural sunlight at noon on a summer day in Freeport, Texas. After 21 hours of exposure, in the presence of NO, less than 5 percent of the DCM initially present had disappeared. Similarly, less than 5 percent disappeared in an NO_2 atmosphere after a 7.5-hour exposure. The effect of varying UV intensity or concentration on the rate of photodecomposition was not investigated. Relative humidity in the photolysis reactor was 35 to 40 percent. Dichloromethane was judged to contribute to oxidant formation to a lower degree than other halogenated compounds investigated, e.g., tetrachloroethylene, trichloroethylene, and vinyl chloride.

Butler et al. (1978) have proposed that OH attack on DCM in the presence of O_2 may result in formation of phosgene ($COCl_2$) via the reaction sequence:

$$CH_2Cl_2 + OH \rightarrow \cdot CHCl_2 + H_2O$$

$$\cdot CHCl_2 + O_2 \rightarrow \cdot CHCl_2O_2$$

$$\cdot CHCl_2O_2 \rightarrow COCl_2 + OH$$

This pathway was suggested to account for a low rate constant of the reaction of OH with DCM in the presence and absence of CO in the test atmosphere. Production of CO_2 was followed.

Chlorine-sensitized photooxidation of DCM in the presence of Cl_2 in dry air resulted in CO and CO_2 as the major carbon-containing products (Spence et al.,

TABLE 3-4. REACTION RATE DATA FOR OH + CH_2Cl_2

k x 10^{-14} cm^3 $molecule^{-1}$ sec^{-1}	°K	Arrhenius Expression	Reference
14.5 ± 2.0	298.5	---	Perry et al., 1976
15.5 ± 3.4	296	---	Howard and Evenson, 1976
11.6 ± 0.5	245 to 375°K	$4.27 \pm 0.63 \times 10^{-12}$ exp [-(1094 ± 81/T)]	Davis et al., 1976
8.7	265	---	
10.4	298	---	Cox et al., 1976
2.7 ± 1	302	---	Butler et al., 1978
---	---	5.2×10^{-12}exp(-1094/T)	NASA,* 1977; National Bureau of Standards, 1978

---

*NASA preferred value; reliability of log k judged to be ± 0.2 at 230°K.

1976). After 5 minutes of irradiation of 20 ppm DCM and 5 ppm Cl_2 in air, 19 ppm CH_2Cl_2 was consumed. The product distribution was: CO (5 ppm); HCl (38 ppm); phosgene (2 ppm); formylchloride (0 ppm); and CO_2 (12 ppm). The product distribution is illustrative of a chain reaction:

$$\cdot CHCl_2O_2 \rightarrow ClO + HCOCl$$

$$HCOCl + Cl \rightarrow HCl + COCl$$

$$COCl + O_2 \rightarrow CO_2 + ClO$$

$$COCl \rightarrow CO + Cl$$

Chlorine-sensitized photooxidation of DCM is not expected to be significant under real atmospheric conditions as Cl will react with species other than halocarbons (Spence et al., 1976).

3.2.5.2 Hydrolysis--The hydrolysis of DCM in natural waters is influenced by acidic and basic conditions. When hydrolyzed in water at temperatures ranging from 80 to 150°C, the hydrolysis in an acidic solution was reported to proceed at a rate corresponding to a measured half-life of 13.75 days (Fells and Moelwyn-Hughes, 1958).

In a review, Radding et al. (1977) reported a maximum hydrolytic half-life of 704 years (100 to 150°C at pH 7); this is in sharp contrast to the aqueous reactivity results found by Dilling et al. (1975) in which the half-life was about 18 months (25°C).

3.2.5.3 Sorption--Dilling et al. (1975) found that DCM could be adsorbed to dry bentonite clay and peat moss when these absorbents were added to a sealed solution containing DCM. However, the DCM which leaches from landfills adsorbs very little to clay, limestone, and/or peat moss, so retention in the soil is unlikely.

3.3 LEVELS OF EXPOSURE

Dichloromethane has been detected in ambient air and in surface and drinking waters at numerous locations throughout the United States.

Average background mixing ratios are approximately 30 to 50 ppt. Ambient air levels of DCM at various locations are shown in Table 3-5. Singh et al. (1979) reported that the average northern hemisphere background mixing ratio is approximately 45 ppt. The Washington State University group reported free troposphere values between 30 and 40 ppt (Cronn et al., 1977; Robinson, 1978; Cronn and Robinson 1979; Grimsrud and Rasmussen, 1975; Rasmussen et al., 1979).

Pellizzari and Bunch (1979) have compiled a list of the sites in the United States at which Pellizzari and coworkers have identified DCM. DCM was sampled by adsorption onto Tenax® GC, coupled with analysis by high resolution GC-MS. The highest level reported in New Jersey was at a waste disposal site in Edison. A level of 360 ppb was measured during an 11 minute sampling period in March 1976. During a 75 minute sampling period (Nov. 1977), 55 ± 0.1 ppb was detected at a site in Staten Island, New York. Longer sampling times (up to 8 hours) were required at sites in Virginia, West Virginia, and Pennsylvania to detect DCM. During a 7 hour and 15 minute period, about 70 ppb were detected at a site in Front Royal, Virginia (Oct. 1977). In the southwest, a level of about 1 ppb was reported at sites in Houston, Texas during a 3-hour sampling period (Oct. 1977). Lower levels over considerably longer sampling periods (up to 24 hours) were reported for sites in Louisiana, e.g., Baton Rouge and Geismar. Sampling during a 48-hour period in Upland, California (Aug. 1977) indicated levels of about 12 ± 9 ppb.

3.3.1 Analytical Methodology

There are four practical methods to measure air concentrations of the halogenated hydrocarbons:

1. gas chromatography with an electron-capture detector (GC-EC);
2. gas chromatography-mass spectrometry (GC-MS);

TABLE 3-5. AMBIENT AIR LEVELS OF DICHLOROMETHANE

Location	Type of site	Date of measurement/ analytical method	Mixing Ratio ppb	Reference
Arizona				
Phoenix	Urban	Apr 23 to May 6, 1979 GC-EC	Max 5.1552 Min 0.0859 Avg 0.8936 ± 0.9886	Singh et al., 1981
California				
Mill Valley	Background subject to urban transport	Jan 11 to 27, 1977 GC-EC	Max 0.087 Min 0.038 Avg 0.055 ± 0.014	Singh et al., 1979
Riverside	Urban	Apr 25 to May 4, 1977 GC-EC	Max 0.473 Min 0.033 Avg 0.111 ± 0.094	Ibid
Badger Pass	High altitude	May 5 to 13, 1977 GC-EC	Max 0.126 Min 0.009 Avg 0.044 ± 0.025	Ibid
Point Arena	Marine coastal	May 23 to 30, 1977 GC-EC	Max 0.102 Min 0.013 Avg 0.045 ± 0.022	Ibid
San Jose	Urban	Aug 21 to 27, 1978 GC-EC	Max 1.920 Min 0.060 Avg 0.401 ± 0.352	Ibid
Point Arena	Marine coastal	Aug 30 to Sept 5, 1978 GC-EC	Max 0.080 Min 0.016 Avg 0.039 ± 0.017	Ibid
Los Angeles	Urban	Apr 9 to 21, 1979 GC-EC	Max 12.0288 Min 0.6014 Avg 3.7511 ± 2.6203	Singh et al., 1981

TABLE 3-5. (continued)

Location	Type of site	Date of measurement/ analytical method	Mixing Ratio ppb	Reference
Panama				
Canal Zone		July 1977, GC-MS	Max - Min - Avg 0.034	Cronn and Robinson, 1979
Southern Hemisphere				
0° to 42°S	Marine	Oct 1976, GC-MS	Avg 0.035 ± 0.003	Robinson, 1978
77° to 90°S	Remote	Jan 1977, GC-MS	Avg 0.034 ± 0.004	
32° to 55°S	Marine	Oct 1977, GC-MS	Avg 0.040 ± 0.002	
77° to 90°S	Remote	Nov 1977, GC-MS	Avg 0.033 ± 0.001	

TABLE 3-5. (continued)

Location	Type of site	Date of measurement/ analytical method	Mixing Ratio ppb	Reference
Oakland	Urban	June 28 to July 10, 1979 GC-EC	Max 2.4058 Min 0.0859 Avg 0.4155 ± 0.3146	Singh et al., 1981
Kansas				
Jetmar	Remote	June 1 to 7, 1978 GC-EC	Max 0.105 Min 0.033 Avg 0.054 ± 0.015	Singh et al., 1979
Nevada				
Reese River	High altitude	May 14 to 20, 1977 GC-EC	Max 0.099 Min 0.015 Avg 0.052 ± 0.022	Singh et al., 1979
North Pacific Ocean				
37°N	Marine	Apr 1977, GC-MS	Avg 0.030 ± 0.008	Cronn et al., 1977
0°N - 33°N	Marine	Oct 1976, GC-MS	Avg 0.033 ± 0.046	Robinson, 1978
Washington				
Pullman	Rural	Dec 1974 to Feb 1975 GC-MS	Max - Min - Avg <0.005	Grimsrud and Rasmussen, 1975
Pullman	Rural	Nov 1975, GC-MS	Avg 0.035	Rasmussen et al., 1979

3. long-path infrared absorption spectroscopy, usually with preconcentration of whole air and then separation of the compounds by gas chromatography (MC-IR); and

4. infrared solar spectroscopy, using the solar spectrum at large zenith angles to obtain great path lengths through the atmosphere.

Each method has advantages and disadvantages and applications for which it is best suited. A major drawback with these techniques is that they do not allow real-time continuous measurements of the halocarbons at ambient levels in the environment.

The two most widely used systems for identifying and measuring trace amounts of DCM that occur in ambient air are (1) gas chromatography-mass spectrometry (GC-MS) and (2) gas chromatography-electron capture detection (GC-EC). Both systems have a limit of detection below 30 parts per trillion (ppt). The GC-EC method has been reviewed by Pellizzari (1974) and by Lovelock (1974). The electron capture detector is specific in that halogenated hydrocarbons are quantitated while non-halogenated hydrocarbons do not respond. Thus, high background levels of non-halogenated hyrocarbons in ambient air or water samples do not interfere with measurements of halogenated hydrocarbons. In a complex mixture in which several compounds may have similar retention times, alteration of the operating parameters of the GC-EC system will usually provide separation of the components. Linearity over a wide concentration range is achieved when the electron capture detector is used in the constant current mode. In this mode, the change in pulse frequency is linearly related to sample concentration. Nitrogen, or a 95 percent argon/5 percent methane mixture, is commonly used as the carrier gas.

3.3.2 Sampling of Ambient Air and Water

The methods used to date to analyze air and water for DCM content have the problems of contamination, absorption, and adsorption. There are four general approaches used to collect samples of air for analysis of trace gas concentration: cryogenic sampling in which liquid helium or liquid nitrogen is used to cool a container to extremely low temperatures; pump-pressured samples, in which a mechanical pump is used without cryogenic assistance to fill a sampler to a positive pressure relative to the surrounding atmosphere; ambient or subambient pressure sampling in which an evacuated container is simply opened and allowed to fill until it has reached ambient pressure at the sampling location; adsorption of selected gases on such adsorbants as molecular sieves or activated charcoal. Contamination and other problems are more serious in low-pressure sampling than in high pressure sampling systems (National Academy of Sciences, 1978).

Water samples are subject to the same possibility of contamination and other problems that exist in air sampling. Particular care must be devoted to the sampling, transportation, and storage of aqueous samples of DCM because of its volatility and the complexity of the samples, especially those containing chlorine or other oxidants. A technique that is often used involves filling and sealing a serum bottle without air space and storing it just above freezing (Kopfler et al., 1976). Water samples generally require additional preparation before analysis. Normally, they may be concentrated by various water analysis techniques but direct aqueous injection is used occasionally in GC analysis.

3.3.2.1 Sampling and Detection in Ambient Air--Several common approaches are used to sample ambient air for trace gas analysis, including (National Academy of Sciences, 1978):

1. Pump-pressure samples: A mechanical pump is used to fill a stainless steel or glass container to a positive pressure relative to the surrounding atmosphere.

2. Ambient pressure samples: An evacuated chamber is opened and allowed to fill until it has reached ambient pressure at the sampling location. If

filling is conducted at high altitude, the sample may become contaminated upon return to ground level.

3. Adsorption on molecular sieves, activated charcoal, or other sorbents.

4. Cryogenic samples: Air is pumped into a container, liquefied, and a partial vacuum is created which allows more air to enter. This method allows for the collection of several thousand liters of air.

Singh et al. (1979) have satisfactorily measured ambient levels of DCM by analysis with GC-EC. Samples were pressurized in stainless steel vessels, then preconcentrated by freezeout on 100/120 mesh glass beads (Tenax® monomer was discontinued since oxygen was found to oxidize the monomer and interfere with electron capture detection). Separation was performed on a column containing 0.2 percent Chromosorb W 1500 80/100 mesh on Carbopack C. A post-column Ascarite water trap was used to remove water prior to electron capture detection. Dual detectors were used to provide a coulometric response.

Harsch et al. (1979) used GC-EC to identify and measure the level of DCM in samples of ambient air. A 500 ml sample was preconcentrated using the freezeout concentration method (Rasmussen et al., 1979). Halocarbons were desorbed onto a stainless steel column packed with 10 percent SF-96 on 100/120 mesh Chromosorb W. The reported detection limit was 26 ppt. Good separation from chlorofluorocarbon 113 (trichlorotrifluoroethane) was reported.

Cronn and Harsch (1979) reported a GC-MS detection limit of 6 ppt for a 500 ml aliquot of DCM. Cronn et al. (1977) also reported a detection limit of 20 ppt for a 100 ml aliquot. Pressurized air samples were separated on a column of Durapack n-octane on 100/120 mesh Porasil C. Cronn and coworkers (1976) have compared GC-MS with GC-EC in terms of precision and sensitivity. In general, GC-MS offered great specificity but could not equal GC-EC in reproducibility for the 11 halocarbons studied. With mass spectrometry, the detection limit for DCM was 9 ppt with a percent standard deviation of 13. The detection limit with temperature programmed GC-EC was 4 ppt and a percent standard deviation of 7.3.

Pellizzari and Bunch (1979) reported an estimated detection limit of 200 ppt using a high resolution GC-MS system in which DCM was first adsorbed onto Tenax® GC. The accuracy of analysis was reported as ±30 percent. The inherent analytical errors are a function of several factors, including: (1) the ability to accurately determine the breakthrough volume; (2) the accurate measurement of the ambient air volume sampled; (3) the percent recovery of DCM from the sampling cartridge after a period of storage; and (4) the reproducibility of thermal desorption from the Tenax® cartridge and its introduction into the analytical system. Oxidation of the Tenax® monomer was not reported.

Difficulties in using a coulometric approach to the GC-EC quantification of DCM in air samples have been reported by Lillian and Singh (1974). These investigators were unable to measure DCM accurately with detectors in series due to a greater-than-coulometric response. Dichloromethane was reported to have a very low ionization efficiency. It was suggested that the observed response is attributed to the products of ionization having greater electron affinities than the reactants.

Cox et al. (1976) reported that polyglycol stationary phase chemically bonded to porous glass (Durasil Low K1) was the only material found to separate DCM from other halocarbons during a GC-EC analysis.

Satisfactory separation and analysis of DCM was reported by Grimsrud and Rasmussen (1975) with a 50-foot SCOT OV-101 column by GC-MS.

When the freezeout concentration method of Rasmussen et al. (1979) was applied to a 500 ml aliquot of air, the detection limit for DCM with GC-EC analysis was 4 ppt and a percent standard deviation of 26.2. The GC column contained 10 percent SF-96 on 100/120 mesh Chromosorb W.

The National Institute for Occupational Safety and Health (NIOSH) method P & CAM 127 (NIOSH, 1974) is recommended for measurement of DCM in samples when the concentration is greater than 0.05 mg in each sample. This method utilizes adsorption on charcoal followed by desorption with carbon disulfide. Analysis is made by gas chromatography with flame ionization detection. The mean relative standard deviation of the method is 8 percent.

Grimsrud and Miller (1979) have reported an improved GC-EC method by which the detector response of DCM is enhanced by the addition of O_2 in the carrier gas. At the highest O_2 doping (5 parts per thousand, ppth) the response of the detector to DCM (960 ppb) was enhanced 57-fold. The enhancement is depicted in Figure 3-1. A constant current electron capture detector was used.

3.3.2.2 Sampling and Detection in Water--A gas purging and trapping method suitable for DCM has been described by Bellar et al. (1979). Water samples are purged by bubbling with helium or nitrogen at 23°C. The halocarbons are adsorbed onto a porous polymer trap as the gas is vented. Quantification is made by GC-MS. Tenax® GC (60/80 mesh) was considered an effective adsorbent for compounds that boil above approximately 30°C. A recommended general purpose column is an 8-foot by 0.1 inch (i.d.) stainless steel or glass tube packed with 0.2 percent Carbowax® 1500 on Carbopack® - C (80/100 mesh). For a sample volume of 5 ml, the range of the limit of detection is 0.1 to 1.0 μg per liter.

3.3.2.2.1 Sample Preservation (Water). Bellar et al. (1979) recommended that water samples be stored in narrow mouth glass vials. Vials are filled to zero head space and covered with a Teflon®-faced silicone rubber septum. Screw caps are suitable seals. The presence of chlorine in water samples has been shown to result in an increase in the concentration of certain halomethanes (not including DCM) upon storage.

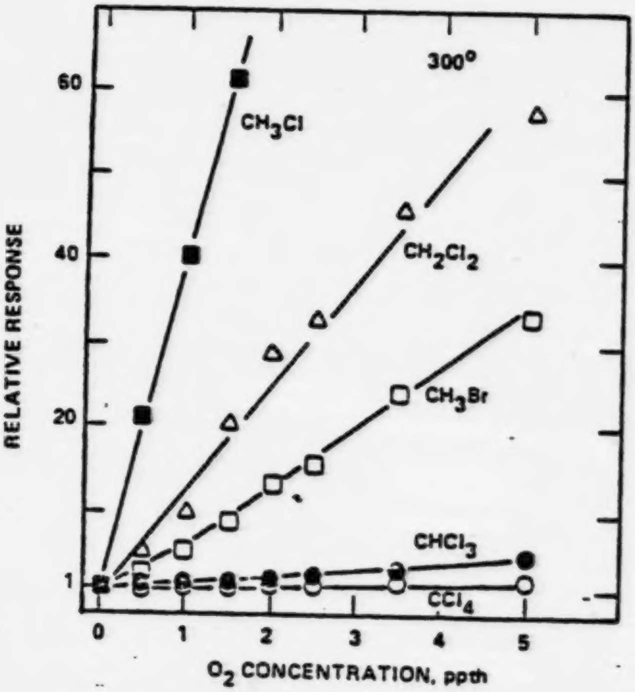


Figure 3-1. The effect of oxygen doping of the carrier gas on the ECD response to several halogenated methanes at a detector temperature of 300° C. From Grimsrud and Miller (1979).

3.3.2.2.2 Soil and Sediment (Water). Dichloromethane has been found in drinking and surface water at a variety of locations in the United States. In a recent National Academy of Sciences report (NAS, 1977) DCM was reported to be formed as a result of chlorination treatment of water.

In a survey for volatile organics in five drinking water supplies, Coleman et al. (1976) found that DCM was common to all cities evaluated (Cincinnati, Miami, Philadelphia, and Ottumwa, Iowa). Concentrations were not reported. Analysis was performed using GC-MS.

In a 1975 survey by the U.S. Environmental Protection Agency (1975a), DCM was detected in 9 of 10 water supplies. Lawrence, Massachusetts, had the highest concentration (1.6 μg/l). A mean concentration of <1 μg/l in finished water was reported in a survey of Region V water supplies (U.S. EPA, 1975b). This survey indicated eight percent of the finished-water supplies contained detectable DCM.

Dichloromethane was not among the major halogenated hydrocarbons detected by Dowty et al. (1975a) in New Orleans drinking water. In another report, DCM was detected in finished waters in the New Orleans area by GC-MS (Dowty et al., 1975b). It was also found in Mississippi River clarifier effluent. The chlorocarbon was sorbed onto poly p-2,6-diphenyl phenylene oxide (35/60 mesh). Vapors were desorbed onto a capillary chromatographic column and quantitated by mass spectrometry. Raw water influent was purified after clarifier treatment (sedimentation and some chemical treatment) to an extent that DCM concentration dropped 32 percent. However, the concentration in finished water increased after chlorination. Dichloromethane also was detected in commercially bottled artesian well water.

A slight increase in DCM concentration in chlorinated finished water was observed by Bellar et al. (1974). Finished water having 2.0 μg/ℓ DCM resulted following complete treatment process of raw river water containing no detectable DCM. Water samples also were collected from various locations in a sewage treatment plant. Before treatment the water contained 2.36 ppb (8.2 µg/l). Before chlorination and after preliminary treatment, the water contained 0.8 ppb (2.9 µg/l). After chlorination the effluent contained 1 ppb (3.4 µg/l). It was concluded that DCM, along with other chlorocarbons, may have formed as a result of the chlorination treatment. The most notable chlorocarbon was chloroform, for which an increase of 7.1 µg/l to 12.1 µg/l was observed subsequent to chlorination. GC-MS analysis was performed using a headspace preconcentration technique.

Dichloromethane was detected at 32 of 204 surface water sites from which samples were collected (Ewing et al., 1977). Sites were located near heavily industrialized river basins across the United States. Concentrations were reported as being greater than 1 ppb (1 µg/l). Samples were collected from July 1975 to December 1976. Of the 204 sites, 91 were among major rivers and 57 were in tidal areas and estuaries. Samples (125 ml) were held at 60°C and stripped. Volatiles were sorbed onto Tenax® GC and desorbed onto Carbowax 1500 columns and analyzed by mass spectrometry.

Dichloromethane was not among the contaminants detected by Sheldon and Hites (1978) in raw Delaware River water collected from August 1976 to March 1977.

Pellizzari and Bunch (1979) reported detecting DCM in untreated Mississippi River water (Jefferson Parish, Louisiana) at a mean concentration of 2.581 µg/l. The highest value reported was 15.8 µg/l. Determinations were made from February 7 to August 5, 1977. A mean concentration of 0.13 µg/l was reported by Pellizzari and Bunch (1979) in tap water from Jefferson Parish. The highest level was 1.1 µg/l.

3.4 ECOLOGICAL EFFECTS

3.4.1 Effects on Aquatic Organisms

Dichloromethane has been tested for acute toxicity in a limited number of aquatic species. The information presented in this chapter focuses upon observed levels that were reported to result in adverse effects under laboratory conditions. Such parameters of toxicity are not easily extrapolated to environmental situations. Test populations themselves may not be representative of the entire species in which susceptibility to the test substance at different lifestages may vary considerably.

Guidelines for the utilization of these data in the development of criteria levels for DCM in water are discussed in the Ambient Water Quality Criteria for Halomethanes (U.S. Enviornmental Protection Agency, 1980b).

The toxicity of DCM to fish and other aquatic organisms has been gauged principally by flow-through and static testing methods. The flow-through method exposes the organism(s) continuously to a constant concentration of DCM while oxygen is continuously replenished and waste products are removed. A static test, on the other hand, exposes the organism(s) to the added initial concentration only. Results from both types of tests are commonly used as initial indications of the potential of substances to cause adverse effects.

3.4.1.1 Effects on Freshwater Species--Results of flow-through and acute static tests with DCM and freshwater species (fish and invertebrates) are shown in Table 3-6.

Alexander et al. (1978) used both flow-through and static methods to investigate the acute toxicity of several chlorinated solvents, including DCM, to adult fathead minnows (*Pimephales promelas*). Studies were conducted in accordance with test methods described by the U.S. Environmental Protection

TABLE 3-6. EFFECTS OF DICHLOROMETHANE ON FRESHWATER SPECIES IN ACUTE TESTS

Species	Type of Test	LC50[*]	EC50[+]	Reference
bluegill (*Lepomis macrochirus*)	static	224,000 µg/l	-	U.S. EPA, 1980a
fathead minnor (*Pimephales promelas*)	static	310,000 µg/l	-	Alexander et al., 1978
fathead minnow (*Pimephales promelas*)	flow-through	193,000 µg/l	-	*Ibid*
Daphnia magna	statis	-	224,000 µg/l	U.S. EPA, 1980a

[*] 96-hour

[+] 48-hour

Agency. No irreversible effects were observed at concentrations below the LD50.

Chronic test data concerning life cycle or embryo-larval tests are not available.

3.4.1.2 Effects on Saltwater Species--Static tests with mysid shrimp resulted in an LC50 value of 256,000 μg/l. There are no chronic data for DCM.

In a 96-hour static test with Sheepshead minnow (*Cyprinodon variegatus*) the LC50 value was 331,000 μg/l (U.S. EPA, 1980a).

3.4.2 Effects on Plants

The 96-hour EC50 values for DCM, based upon chlorophyll *a* and cell numbers of the freshwater alga, *Selenastrum capricornutum*, were above the highest test concentration (662,000 μg/l).

The 96-hour EC50 value, based on chlorophyll *a* and cell numbers of the saltwater alga, *Skeletonema costatum*, was above the highest test concentration (662,000 μg/l) (U.S. EPA, 1980a).

3.5 CRITERIA, REGULATIONS AND STANDARDS

Permissible levels of DCM in the working environment have been established in various countries. The U.S. Occupational Safety and Health Administration (OSHA) health standard requires that a worker's exposure to DCM at no time exceed 500 ppm (1,737 mg/m^3) time-weighted average in any 8-hr work day of a 40-hr week, with an acceptable ceiling concentration of 1000 ppm (3,474 mg/m^3), that should not exceed 2,000 ppm (6,948 mg/m^3) for more than 5 minutes in any 2 hours. The American Conference of Government Industrial Hygiene (ACGIH) threshold limit value (TLV) for inhalation exposure of 200 ppm (695 mg/m^3), proposed for prevention of narcotic effects or liver injury and for protection against excessive carboxyhemoglobin formation, has recently been lowered to 100 ppm (347 mg/m^3). The 8-hour time-weighted average value in the Federal Republic of Germany is

500 ppm; in the German Democratic Republic and Czechoslovakia, 144 ppm; and in Sweden, 100 ppm. The acceptable ceiling concentration in the USSR is 14 ppm (49 mg/m^3). The National Institute for Occupational Safety and Health (NIOSH) has recommended that occupational exposure to DCM not exceed 75 ppm (261 mg/m^3), determined as a time-weighted average for up to a 10-hour work day of a 40-hour week, in the absence of exposure to carbon monoxide above a time-weighted average of 9 ppm for up to a 10-hr work day.

The U.S. Environmental Protection Agency water quality criteria are scientific assessments of ecological effects and human health effects for incorporation into water quality standards (U.S. EPA, 1980a). The available data for halomethanes including dichloromethane indicate the acute toxicity to freshwater aquatic life occurs at concentrations as low as 11,000 μg/l; and acute and chronic toxicity to saltwater aquatic life occurs at concentrations as low as 12,000 and 6,400 mg/l, respectively. Toxicity would be expected to occur at lower concentrations among species that are more sensitive than those tested. A decrease in algae cell numbers occurs at concentrations as low as 11,500 μg/l.

Because positive results for mutagenic endpoints correlate with positive results in *in vivo* bioassay for oncogenicity, mutagenic data for the halomethanes suggest that several of the compounds might be carcinogenic. The U.S Environmental Protection Agency Carcinogen Assessment Group is currently deferring a final cancer assessment of DCM until further information becomes available. However, in the Ambient Water Quality Criteria for Halomethanes (U.S. EPA, 1980a) it was noted that for the maximum protection of human health from the potential carcinogenic effects due to exposure to DCM, or combinations of this chemical with chloromethane, bromomethane, bromodichloromethane, tribromomethane, dichlorodifluoromethane, and/or trichlorofluoromethane, through ingestion of contaminated water and contaminated aquatic organisms, the ambient water concentration should be zero based on the assumption of no threshold for these chemicals. However, since zero level may not be attainable the levels that may result in incremental increase of cancer risk over the lifetime are estimated at 10^{-5}, 10^{-6}, and 10^{-7}. The corresponding recommended criteria, based upon data for chloroform, are 1.9 µg/l, 0.19 µg/l, and 0.019 µg/l, respectively. If the above estimates are made for consumption of aquatic organisms only, excluding consumption of water, the levels are 157 µg/l, 15.7 µg/l, and 1.57 µg/l, respectively. Estimates for water consumption only were not made.

In cases such as halomethanes where one criterion is derived for an entire class of compounds, the Agency does not state that each chemical in the class is a carcinogen. The intended interpretation of the criterion is that the risk is less than 10^{-5} whenever the total concentration of all halomethanes in water is less than the criterion. In a hypothetical case where all of the halomethanes in a sample are non-carcinogenic, the criterion would be too strict; however, this situation seldom occurs. In most cases where halomethanes are detected, a mixture of compounds occurs and in calculation of the criterion the assumption is made that all components have the same carcinogenic potency as chloroform.

The derived water quality criterion based on noncarcinogenic risks, assuming a daily water intake of 2 liters and the consumption of 6.5 g of fish and shellfish per day (bioconcentration factor 0.91), would be 12.4 mg/l.

The available data for halomethanes indicate that acute and chronic toxicity to saltwater aquatic life occur at concentrations as low as 12,000 and 6,400 µg/l, respectively, and would occur at lower concentrations among species that are more sensitive than those tested. A decrease in algal cell numbers occurs at concentrations as low as 11,500 µg/l.

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4. METABOLIC FATE AND DISPOSITION OF DICHLOROMETHANE

4.1 ABSORPTION, DISTRIBUTION, AND ELIMINATION

4.1.1 Oral, Dermal, and Lung Absorption

Dichloromethane (DCM) is a colorless liquid with a pleasant smell and physical properties that make it an excellent organic solvent for many uses in industry and in certain consumer products. Because of its relatively high vapor pressure at room temperatures (350-400 torr), DCM is readily absorbed into the body following inhalation. Although it can be detected in air by its odor at about 200 ppm (May, 1966; Leonardos, 1969), the great majority of severe poisonings from this solvent occur from inhalation exposure; reports of poisoning by oral ingestion are rare (Llewellyn, 1966; Stewart and Hake, 1976; Friedlander et al., 1978). While absorption through the intestinal mucosa after oral ingestion appears to be rapid and complete, total recovery has occasionally followed the swallowing of quite large doses (Roberts et al., 1976).

The limited information available indicates that absorption of DCM through the skin from direct liquid contact or by immersion of hands or arms is a slow process. Early animal studies by Schutz (1958) showed that DCM does penetrate the skin and can be absorbed into the body by this route. Schutz exposed the shaved skin of rats to direct liquid contact for up to 20 minutes and found that only 2 minutes of exposure produced kidney damage probably through solvent action leading to decreased urine formation and the appearance of blood in the urine. Stewart and Dodd (1964) attempted to quantify the rate of absorption through human skin by immersing the thumbs of volunteers into liquid DCM and then determining the appearance and concentration of DCM in the breath. An estimate of the amount entering the body was made by comparison with breath concentrations obtained following controlled inhalation exposures. They concluded from breath analysis after 10 minutes of immersion (1.4 to 2.4 ppm, 4.86 to 8.34 mg/m^3) and after 30 minutes of immersion (2.4 to 3.6 ppm, 8.34 to 12.51 mg/m^3) that DCM is very slowly absorbed. They also determined that although the amount absorbed also depends on the skin area involved, the slow rate of absorption would be unlikely to result in toxic quantities of DCM being taken into the body from direct contact with the skin of the hands and forearms. Indeed, immersion in DCM was found to be accompanied by excruciating pain within a few minutes that would no doubt serve as an effective deterrent.

In spite of the long use of DCM as a general industrial solvent spanning at least six decades (Lehmann and Schmidt-Kehl, 1936), and although its narcotic and anesthetic properties have been known to clinical medicine for over 50 years (Bourne and Stekle, 1923), few controlled and definitive studies have been made of its pharmacokinetics and metabolism in humans exposed to low concentrations. Most available information is derived from studies appearing within the last 8 years, stimulated by the resurgence of interest in DCM following the demonstration of its metabolism to carbon monoxide (CO) (Stewart et al., 1972). Of interest also is the question of the contribution of this metabolic transformation to the well-known neurotoxicity and cardiotoxicity of DCM. These studies have been greatly facilitated by the development of gas chromatographic methods for the determination of DCM and its metabolites in alveolar air and body fluids and tissues (Latham and Potvin, 1976; DiVincenzo et al., 1971).

4.1.2 Pulmonary Uptake and Tissue Distribution

Dichloromethane is appreciably more water soluble (2 g per 100 ml) and less lipid soluble than its congeners, chloroform and carbon tetrachloride.

At 37°C, DCM has a partition coefficient (olive oil/water) of 180 (Lehmann and Schmidt-Kehl, 1936; Morgan et al., 1972). Morgan et al. (1972) suggest, from correlation of lipid solubility and toxic effects of halohydrocarbons, that compounds with lower lipid partition coefficients may not achieve sufficient levels in tissues to produce acute liver and kidney damage like chloroform and carbon tetrachloride.

Because of its water and lipid solubility and the very large lung alveolar surface area, inhaled DCM rapidly equilibrates across the alveolar endothelium. The blood/air and water/air Ostwald coefficients of 7.9 and 7.2 at 37°C, respectively, indicate that DCM is largely dissolved in plasma and cellular water rather than lipid components of blood (Morgan et al., 1972; Lindqvist, 1978). Therefore, DCM probably distributes throughout the body water and has been detected in urine (MacEwen et al., 1972; Divincenzo et al., 1972) and breast milk (Vozovaya et al., 1974). Because of its lipid solubility, it distributes to all body tissues and cellular lipids. It readily crosses the blood-brain barrier even at relatively low vapor exposure concentrations as evidenced by its impairment of manual and mental performance at 500 ppm (1737 mg/m^3) (Winneke and Fodor, 1976). It also crosses the placenta and may affect fetal development (Schwetz et al., 1975). Tissue concentrations of DCM increase with exposure concentration and duration and, for any given tissue, are dependent also on the largely unknown tissue partition coefficients. Engstrom and Bjurstrom (1977) determined that the Ostwald coefficient for subcutaneous adipose tissue from human buttocks is 51 at 60°C, a value which indicates that the partition coefficient for this tissue/blood may be about 7 at body temperature.

The magnitude of DCM uptake into the body (dose, burden) primarily depends on several parameters: inspired air concentration, pulmonary ventilation, duration of exposure, and the rates of diffusion into and solubility in the various tissues. The concentration of DCM in alveolar air, in equilibrium with pulmonary venous blood content, approaches a minimum difference with the concentration in the inspiratory air until a steady-state condition is reached. After tissue and total body equilibrium is reached during exposure, uptake is balanced by elimination through the lungs and by other routes, including metabolism. The difference between alveolar and inspiratory air concentrations, together with the ventilation rate (about 6 l/min at rest), provides a means of calculating uptake during exposure:

$$Q = (C_{insp} - C_{alv})\ \dot{V} \cdot T$$

where Q is the quantity absorbed; C, the concentration in mg/l; $\dot{V}$, alveolar ventilation rate in l/min; T, duration of exposure in minutes. The percent retention is defined as $(C_{insp} - C_{alv})/C_{insp} \times 100$, and % retention x quantity inspired $(\dot{V} \cdot T \cdot C_{insp})$ is equal to uptake.

Figure 4-1 illustrates the overall time-course of absorption and elimination during and after a 2-hour inhalation exposure of 100 ppm (347 mg/m^3) DCM for a 70-kg man. During exposure, the alveolar air concentration of DCM can be described by an exponentially rising curve with three components. At the beginning of exposure an initial rapid rate of uptake occurs [0 to 50 ppm (0 to 173 mg/m^3) alveolar air], followed by a second slower uptake [50 to 65 ppm (173 to 208 mg/m^3) alveolar air] and finally a very slow rate as equilibrium is approached at 70 ppm (243 mg/m^3) alveolar air concentration. The total quantity of DCM absorbed and retained in the body during exposure is represented by the area between the alveolar and inspired environmental air concentration curves. Complete equilibrium or steady-state conditions are not attained by the end of the 2-hour exposure to DCM as shown by the slowly rising alveolar concentration curve. The three uptake compartments of the

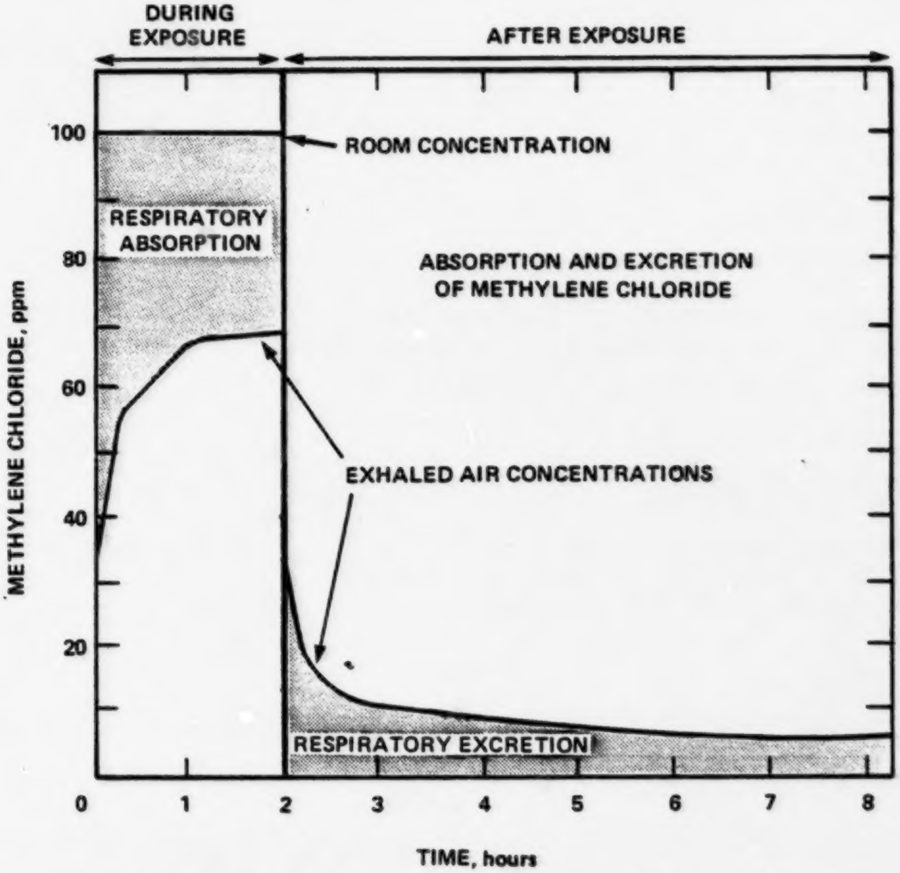

Figure 4-1. Inspired and expired air concentrations during a 2 hr, 100 ppm inhalation exposure to DCM for a 70 kg man, and the kinetics of the subsequent pulmonary excretion. From Riley et al., 1966.

exponential alveolar curve correspond to equilibrium attained by first order passive diffusion of DCM from blood first through a vessel-rich group (VRG) of tissues with high blood flow (VRG: brain, heart, kidneys, liver, endocrine and digestive systems), then more slowly through the lean body mass (muscle group, MG: muscle and skin) and lastly through adipose tissues (fat group, FG). With termination of exposure, blood and alveolar air DCM concentrations decline in parallel in an exponential manner with three components of pulmonary elimination and desaturation from VRG, MG, and FG body compartments. The area under the alveolar elimination curve in Figure 4-1 is proportional to the quantity of DCM absorbed during the 2-hour exposure.

The retention of DCM as a percentage of inspired air concentration is independent of that concentration at equilibrium. Retention values for DCM, reported by different investigators, are shown in Table 4-1. These values have a large range and vary with duration of exposure. As expected, the values are higher for short exposures of 20 to 30 minutes (75 percent retention). Variation in the values is also due, in part, to differences in body weights of the subjects and differences in body composition (proportion of adipose to lean mass). For exposures greater than one hour, the mean retention approximates 42 percent of uptake of DCM or approximately 125 mg/hr for an exposure of 100 ppm, assuming a resting ventilation rate of 6 l/minute.

The quantity (dose) of DCM absorbed into the body is, for short exposures, theoretically directly proportional to the concentration of DCM in the expired air. This relationship has been confirmed experimentally (Lehmann and Schmidt-Kehl, 1936; Astrand et al., 1975). The body burden of DCM also increases with exposure duration and with physical activity (increased ventilation and cardiac output) at a given inhaled air concentration (Engstrom and Bjurstrom, 1977; Astrand et al., 1975). Astrand et al. (1975) found that physical activity

TABLE 4-1. ABSORPTION OF DCM BY HUMAN SUBJECTS (SEDENTARY CONDITIONS)

Investigator	Inhalation concentration, ppm	Exposure, hr	Retention, %
Lehmann and Schmidt-Kehl, 1936	662	0.30	74
	806	0.50	75
	1,152	0.50	72
	1,181	0.50	70
Riley et al., 1966	44-680	2.00	31
DiVincenzo et al., 1972	100	2.00	53
	100	4.00	41
	200	2.00	51
Astrand et al., 1975	250	0.50	55
	500	0.50	55
Engstrom and Bjurstrom, 1977	750	1.00	34

during exposure to 250 and 500 ppm (869 and 1,737 mg/m^3) DCM for 0.5 hour decreased retention from 55 percent in a resting state to 40 percent during activity, but doubled the amount of DCM absorbed because of a three-fold increase of ventilation rate (6.9 to 22 l/minute).

The quantity of DCM absorbed is dependent also on body weight and fat content of the body. Engstrom and Bjurstrom (1977) showed that for an exposure to 750 ppm (2,606 mg/m^3) for 1 hour the amount of DCM absorbed into the body was directly proportional to body weight and to body fat content expressed as a percentage of body weight. Obese subjects (avg. body fat 25 percent of body weight) absorbed 30 percent more DCM than lean subjects (avg. body fat 8 percent of body weight). Biopsy analysis of subcutaneous adipose tissue of obese subjects revealed concentrations of 10.2 and 8.4 mg DCM/kg tissue weight at 1 and 4 hours postexposure, respectively. These concentrations, although lower than found in adipose tissue of lean subjects, represented a greater total storage amount in obese subjects since their total fat stores were greater. Significant amounts of DCM were found in adipose tissue (1.6 mg/kg) of obese subjects 22 hours after exposure, indicating that elimination of DCM from the FG compartment proceeds at a slow rate, and that accumulation, particularly in obese people, may occur with repeated daily exposure. This conclusion is supported by the findings of Savolainen et al. (1977), who exposed rats chronically to DCM [200 ppm (695 mg/m^3), 6 hours daily, for 5 days] and determined DCM concentrations in perirenal fat and in other tissues. Their data, shown in Table 4-2, indicate that significant amounts of DCM remained in perirenal fat 18 hours after the previous exposure of day 4, and markedly increased further with a 6-hr exposure on day 5.

TABLE 4-2. TISSUE CONCENTRATIONS OF DCM IN RATS EXPOSED TO 200 PPM FOR 4 DAYS FOR 6 HR DAILY*

Exposure on the 5th day, hr	nmoles/g tissue wet weight ± S.D.				
	Cerebrum	Cerebellum	Blood	Liver	Perirenal fat
0	-	-	-	-	113 ± 29
2	73 ± 20	57 ± 20	90 ± 10	85 ± 2	526 ± 94
3	119 ± 33	86	79 ± 3	82 ± 1	537 ± 33
4	57 ± 8	95 ± 8	120 ± 10	101 ± 13	608 ± 58
6	83	90	100 ± 1	83 ± 10	659 ± 77

*Derived from Savolainen et al. (1977).

Previous work by DiVincenzo et al. (1972) in man, and Carlsson and Hultengren (1973) in rats, had indicated that little uptake by adipose tissue occurs. However, in their studies single, short exposure periods of 2 hours were used, and of the smaller amounts absorbed, 95 percent was probably accommodated in VRG and MG compartments since the FG compartment receives only 5 percent of the cardiac output.

The blood concentration of DCM during inhalation and in the elimination phase after exposure parallels alveolar DCM concentration. This predictable relationship is defined by the solubility of DCM. Astrand et al. (1975) showed that for men exposed to 250 and to 750 ppm (869 and 2,606 mg/m^3) DCM for 1.5 hour, the arterial blood (mg/l) : alveolar air (mg/l) concentration ratios were constant and averaged 10.3 and 11.1, respectively, over three-fold changes in alveolar concentrations. These *in vivo* Ostwald coefficients are in good agreement with the value (8) found by Lindqvist (1978) for blood/air at 37°C *in vitro*. MacEwen et al. (1972) determined the DCM blood concentration in dogs continuously exposed for 16 days to 1,000 and 5,000 ppm DCM. Blood levels found were 36 and 183 mg/l, respectively, in direct proportion to exposure concentration. Total equilibrium can be assumed to have occurred in these animals. Ostwald coefficients of 10.4 and 10.5 for the two exposure concentrations are in close agreement with the above values noted in man. Similar values can be calculated from the data of Latham and Potvin (1976); Figure 4-2 shows the proportional relationship they found in rats between blood and inspired air concentrations over a range of 1,000 to 8,000 ppm (3.474 to 27.792 g/m^3).

4.1.3 Elimination

The evidence in the literature indicates that any significant elimination of DCM is only by the pulmonary route. MacEwen et al. (1972) found DCM in urine collected from dogs 6 and 24 hours after a 5,000 ppm inhalation exposure. Analysis revealed 51 and 33 mg/l urine, respectively, demonstrating that there is some excretion of DCM by this route. However, these amounts represent less

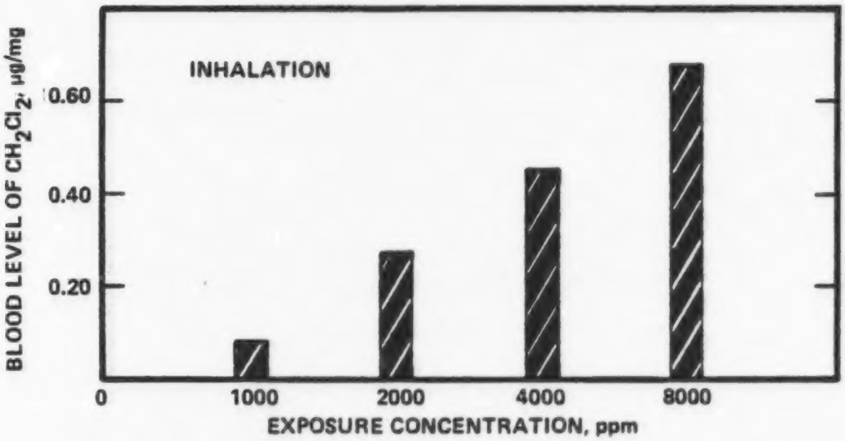

Figure 4-2. DCM venous blood levels in rats immediately after a single 6-hour inhalation exposure to various concentrations of DCM. From Latham and Potvin, 1976.

than 2 percent of the probable body dose. DiVincenzo et al. (1972) found an average of 22.6 μg of DCM excreted in the urine of four subjects during a 24-hour period after exposure to 100 ppm (347 mg/m^3) for 2 hours, and an average of 66.4 μg for seven subjects exposed at 200 ppm (695 mg/m^3) for 2 hours. These very small amounts are less than one percent of the expected body retention of DCM.

Figure 4-1 also shows schematically the time-course of pulmonary elimination of DCM after exposure. The parameters of elimination equilibration of the body are the same as those of assimilation equilibration. After termination of exposure, DCM immediately begins to be eliminated from the body via the lungs. Alveolar air equilibrates with pulmonary venous blood whose concentration becomes a function of the first order diffusion of DCM from tissues, the arterial blood flow/tissue mass, and the relative solubilities of DCM in tissues. Figure 4-1 shows that alveolar DCM concentration follows an exponential decay curve with three major components reflecting de-saturation of the VRG, MG, and FG compartments, respectively. The half-times of elimination of DCM from these compartments have not been firmly established. DiVincenzo et al. (1972), who exposed subjects to 100 ppm and 200 ppm (347 and 695 mg/m^3) for 2- and 4-hour periods, felt that "very little vapor" reached the fat stores and muscle tissues under these conditions. They found DCM to have a half-time value in blood of 40 minutes following 2 hours of exposure, and prolonging exposure to 4 hours had no significant effect on the half-time. However, the low exposure concentrations, combined with the poor recovery of their gas chromatographic method for blood DCM, suggests that these investigators were not able to follow the complete blood decay curve. Riley et al. (1966) measured expired air concentrations after termination of exposure and found half-times of 5 to 10 minutes for the VRG compartment, 50 to 60 minutes for the MG Compartment, and 400 minutes for the FG compartment. Morgan et al. (1972), using isotopically labeled DCM, estimated the half-time of DCM in the VRG compartment as 23 minutes. Engstrom and Bjurstrom (1977) reported the half-time of the MG compartment for lean subjects as about 60 minutes with a longer value in obese subjects. For both lean and obese subjects, they found, by biopsy, residual concentrations of DCM in adipose tissue nearly 24 hours after exposure, suggesting that the half-time of elimination from the FG compartment is fairly long. From the postexposure alveolar concentration curves prepared by Stewart et al. (1976a) for subjects exposed to inhalation concentrations of 50 to 500 ppm (173 to 1,736 mg/m^3) DCM for 1 to 7.5 hours per day for 5 successive days, the half-time of elimination for MG and FG can be estimated as 60 to 80 minutes, and 240 minutes, respectively. Thus, the best guess from these studies for the half-times of elimination from the vessel rich tissues (VRG), the muscle mass (MG), and the adipose tissue (FG) are 8 to 23 minutes, 40 to 80 minutes, and 6 to 6.5 hours, respectively. The long half-time of elimination of the adipose tissue compartment, together with reports that DCM remains in this compartment 24 hours after single and chronic exposures indicates that DCM may very slowly accumulate in body fat with long daily exposures to high air concentrations. The risk of accumulation might be expected to be greater for obese persons.

4.2 DCM BIOTRANSFORMATION

DCM is known to be metabolized in man to carbon monoxide (CO) primarily by the liver. The CO production results in an elevation of blood carboxyhemoglobin (COHb) content, from which CO dissociates at the lung and is then eliminated. Experiments in animals suggest that the metabolism is limited by hepatic enzyme saturation at low tissue concentrations of DCM. The extent of metabolism in man, as a percentage of retained DCM dose, has not been determined by experiment, although estimates from extrapolation of blood COHb levels during and after inhalation exposure indicate a metabolism of less than 10 percent of the body dose. The details of hepatic biotransformation of DCM and the kinetics of blood COHb formation and elimination are reviewed below.

Before 1972, most of the absorbed dose of DCM was thought to be excreted unaltered in exhaled air, while a small amount was found in the urine (MacEwen et al., 1972; DiVincenzo et al., 1972; Heppel et al., 1944). Metabolism of DCM to CO was not known to occur. In 1972, however, Stewart et al. discovered that the COHb concentration in blood increased in persons exposed to 200 to 1,000 ppm (695 to 3,474 mg/m^3) DCM for 1 to 2 hours. The COHb levels continued to rise beyond cessation of exposure and decreased more slowly than when similar levels of COHb were induced by breathing CO. Stewart and his associates (1972,1972a) proposed that CO was the end product of DCM metabolism. In the following year, Fodor et al. (1973) showed that COHb blood levels from DCM exposure were further elevated by concomitant exposure to diiodomethane and dibromomethane, indicating that these dihalomethanes are also metabolized to CO. At first this unique halocarbon metabolism was not generally accepted, since the increased COHb levels might well reflect a change in the rate of endogenous CO production or excretion that is associated with heme degradation by the microsomal hepatic heme oxygenase system in man (Coburn, 1973; Tenhunen et al., 1969). Stewart et al. (1972,1972a), however, observed no evidence of an enhanced metabolism of hemoglobin in their subjects exposed to DCM, and the subjects did not excrete increased amounts of urobilinogen in their urine either during or after exposure.

A second hypothesis put forth to explain the origin of the excess COHb postulated a DCM-induced conformational change in hemoglobin that increased CO affinity. Hence more COHb would be formed, with a longer biological half-life, from endogenous and exogenous CO sources at the same ambient concentrations. Settle (1971) had shown that xenon and, to a much larger degree, cyclopropane, bind to myoglobin and increase CO affinity. Following this observation, Nunes and Schoenborn (1973), using X-ray diffraction, demonstrated that DCM also binds to sperm whale myoglobin and suggested that this binding might increase CO affinity. Settle (1975) then investigated the CO binding to human hemoglobin (Hb). He observed that CO binding to human Hb _in vitro_ at 37°C is increased in the presence of DCM (1,000 ppm, 3,474 mg/m^3). The amount of COHb formed at a given CO concentration is doubled. Also, measurements of the P_{50} values of Hb (partial pressure of O_2 or CO at which 50 percent saturation occurs at 37°C) in the presence and absence of DCM (1,000 ppm) indicated a six-fold increase in CO affinity. From these observations, Settle (1975) suggested that the increased COHb seen _in vivo_ may be due to an increase in CO affinity and not to metabolism. More recently, Collison et al. (1977) have determined the Haldane affinity constant for CO for both human and rat blood equilibrated at 37°C with air containing only CO and CO plus DCM (10,000 ppm). No difference in the Haldane constants for human blood (mean value, 227) or rat blood (mean value, 179) was found. Negative results were also obtained when the absolute affinity of CO was measured in a nitrogen-DCM atmosphere. Dill et al. (1978) re-determined the P_{50} value for human and rat blood in the presence and absence of CO (2,500 ppm) and DCM (800 ppm). DCM was found to have no effect on the P_{50} values.

The biotransformation of DCM to CO and CO_2 has now been confirmed by several metabolism studies. The hepatic metabolism of DCM has been unequivocally shown to be the origin of the CO responsible for increased COHb blood concentrations. Independently, several groups have shown by administering

^{13}C-DCM or ^{14}C-DCM to rats that labeled CO subsequently appears in COHb with essentially the same specific activity (Carlsson and Hultengren, 1975; Miller et al., 1973; Kubic et al., 1974; Zorn, 1975). Furthermore, Fodor and coworkers (1973,1976) demonstrated that rats exposed to CO and DCM, singly and then in combination, effected an additive increase in blood COHb levels (Table 4-3). In addition, many investigators have shown a dose-response relationship between injected or inhaled DCM and increased blood COHb levels in both experimental animals and men (Figure 4-3) (DiVincenzo et al., 1972; Astrand et al., 1975; Fodor et al., 1973; Ratney et al., 1974; Stewart et al., 1973, 1976; Forster et al., 1974; Roth et al., 1975; Ciuchta et al., 1979; Hake et al., 1974).

The metabolism of halogenated hydrocarbons to CO is apparently unique to the dihalomethanes. It is not observed with chloroform, carbon tetrachloride, methyl chloride, methyl iodide, trichlorofluoromethane, dichlorodifluoromethane, carbon disulfide, formaldehyde, formic acid or methanol (Miller et al., 1973; Kubic et al., 1974; Fodor and Roscovanu, 1976; Rodkey and Collison, 1977). Fodor and Roscovanu (1976) state, without giving data, that chloroform, bromoform and iodoform are metabolized to CO in the rat, thus increasing blood COHb levels, although this has not been the observation of other investigators. According to Fodor and Roscovanu (1976), of the dihalomethanes, the bromo-, iodo-, and bromo-iodo-halides are more extensively metabolized to CO than is DCM (Figure 4-4), thus increasing COHb to a significantly greater extent than DCM (Miller et al., 1973; Kubic et al., 1974; Fodor and Roscovanu, 1976; Rodkey and Collison, 1977).

4.2.1 Magnitude of DCM Metabolism

Balance studies with isotopically labeled DCM to determine the extent of biotransformation have not been attempted in man; however, several groups have

TABLE 4-3. BLOOD CARBOXYHEMOGLOBIN CONCENTRATIONS OF RATS EXPOSED TO CO AND DCM BY INHALATION

DCM	CO	COHb
Exposure concentration ppm		%Saturation
100	(0.5 - 2)*	6.2
1000	(0.5 - 2)*	12.5
0	100	10.9
100	100	16.4
1000	100	19.0

*Ambient air CO concentration. Data abstracted from Fodor et al., 1973.

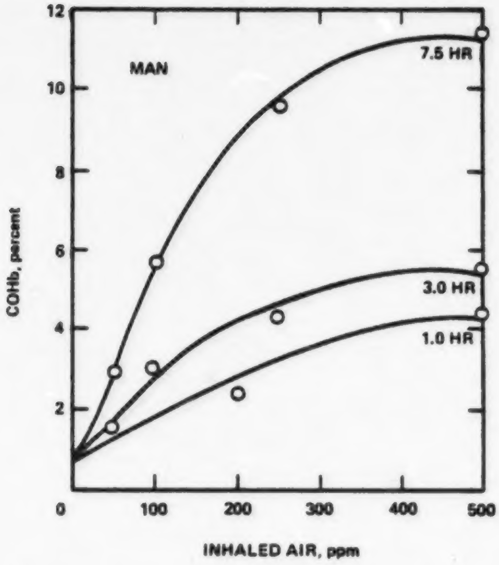

Figure 4-3a. Carboxyhemoglobin concentrations in male nonsmokers exposed to increasing concentrations of DCM for 1, 3, or 5 hr per day for 5 days. Pre-exposure values averaged 0.8%, but with 3 and 7.5 hr exposures were above this baseline value on the mornings following exposure. Data derived from Stewart and his associates (1972, 1973, 1974).

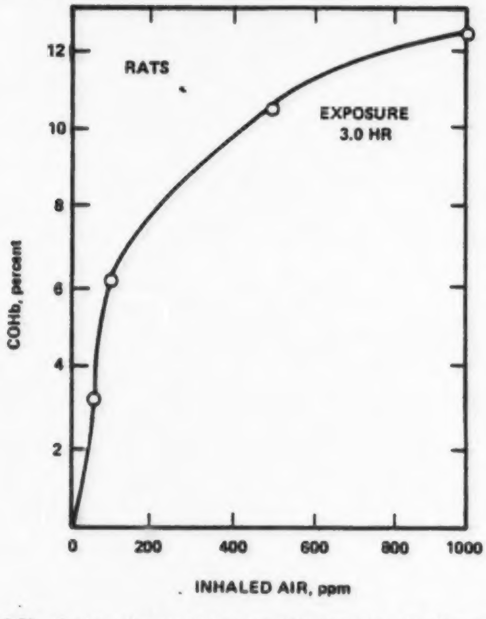

Figure 4-3b. Carboxyhemoglobin concentrations in rats after exposure to increasing inhaled concentrations of DCM for single exposures of 3 hr. The values are corrected for pre-exposure COHb concentration and calculated from the data of Fodor et al., 1973.

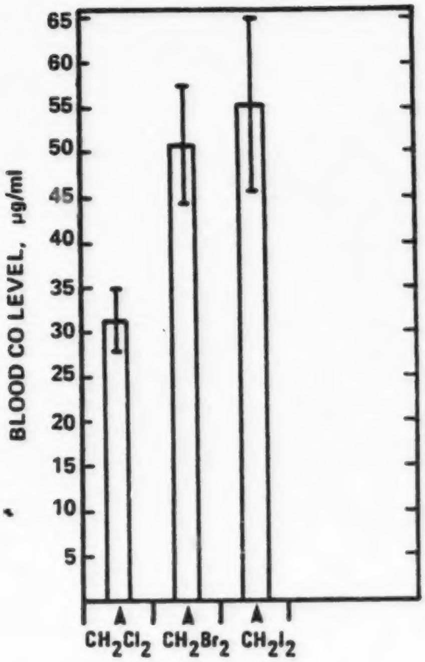

Figure 4-4. Blood CO content of rats after 3-hour inhalation exposure with 1000 ppm dichloromethane, dibromomethane, and diiodomethane, respectively. From Fodor and Roscovanu, 1976.

carried out such investigations in experimental animals. DiVincenzo and Hamilton (1975) provided the first information on the extent to which DCM is metabolized in rats. These investigators injected rats intraperitoneally with ^{14}C-DCM in corn oil and determined fate and disposition of radioactivity in exhaled air, urine, feces and carcass 2, 8, and 24 hours after single doses ranging from 412 to 930 mg/kg. Volatile compounds in exhaled air were collected, identified and quantified by GC and radiotracer assay. Recovery of radioactivity was essentially 100 percent 24 hours after administration. About 98 percent of total radioactivity was eliminated in exhaled air, and less than 2 percent in urine or feces (Table 4-4). Some 90 percent of injected DCM was eliminated unmetabolized in exhaled air. Most of this elimination (95 percent) occurred within 2 hours. Only 2 percent of the dose was metabolized to CO, 3 percent to CO_2, and 1.5 percent to an unidentified volatile compound (Table 4-4). These results indicate that less than 7 percent of the dose is metabolized in the rat.

Rodkey and Collison (1977) considered that the small proportion of DCM found by DiVincenzo and Hamilton (1975) to be metabolized could be due to the high dose of DCM used; i.e., metabolic transformation of DCM may be limited by DCM excretion being more rapid than metabolism. These investigators carried out balance studies with small doses of ^{14}C-DCM administered to rats (17 mg/kg) by either inhalation or intraperitoneal injection. The animals were placed in a closed rebreathing system with traps for CO_2 and CO after conversion to CO_2 by passing through a catalyst bed of Hopcalite®. Some 76 percent of ^{14}C-radioactivity was recovered as ^{14}CO (46.9 percent) and $^{14}CO_2$ (28.9 percent). The remaining 24 percent unaccounted for could have been exhaled as unchanged ^{14}C-DCM, since no radioactivity was recovered in carcass tissues. Their results also showed that DCM is directly metabolized to CO without

TABLE 4-4. FATE AND DISPOSITION OF ^{14}C-DCM IN RATS (412-430) INJECTED INTRAPERITONEALLY*

	As % of dose (averages)		
	2 hr	8 hr	24 hr
Breath			
unchanged ^{14}C-DCM	84.5	94.0	91.5
^{14}CO	0.14	1.43	2.15
$^{14}CO_2$	0.55	1.53	3.04
unidentified ^{14}C	0.40	0.80	1.49
	85.59	97.76	98.18
Urine			
unidentified ^{14}C	<0.01	<0.01	1.06
Feces			
unidentified ^{14}C	<0.01	<0.01	0.07
Carcass			
unidentified ^{14}C (mainly in liver)	3.09	2.06	1.53

*Derived from DiVincenzo and Hamilton, 1975.

isotopic dilution. The extent of the conversion was surprisingly great at this low dose, and independent of the mode of administration. For each mole of DCM metabolized, about 0.5 mole of CO and 0.3 mole of CO_2 were produced.

Rodkey and Collison (1977), in a second experiment, investigated the relationship between dose and extent of metabolism. DCM was administered to rats by intraperitoneal injection or by vaporization of the dose in their closed rebreathing system without a CO trap. Doses from 6.8 to 69 mg/kg were given. CO production and DCM disappearance were calculated from the change in gas phase composition as determined by GC. A control period was used to measure the endogenous rate of CO production. When DCM was added to the system there was an immediate increase in the initial rate of CO production (about 35 times endogenous for all doses), and a rapid disappearance of DCM from the gas phase (90 percent in 30 minutes). CO continued to be produced for more than 2 hours after nearly complete disappearance of gaseous DCM. Figure 4-5 shows the rates of CO production for various doses of DCM. The initial rates [about 25 μmole/hr/kg body weight (b.w.)] are similar for all doses, but for lower doses they progressively decrease after 1 to 2 hours to the endogenous rate as the DCM dose is metabolized. For a very high dose of DCM (68 mg/kg), CO was produced at a nearly constant rate over a 6-hr period (COHb, 44 percent). These observations suggest a saturation of the metabolizing enzymes even at the lowest dose (6.8 mg/kg), giving initially zero-order kinetics followed by first-order kinetics as the DCM concentration in the inhaled air is decreased below enzyme saturation. The total amount of CO produced was related to the dose of DCM given. For lower doses, the moles of CO produced per mole of DCM were similar and averaged 0.48; at high DCM dose (68 mg/kg), the ratio was 0.62 after 10.5 hours of exposure, suggesting that substrate-induced enzyme formation may occur with long exposure to high doses

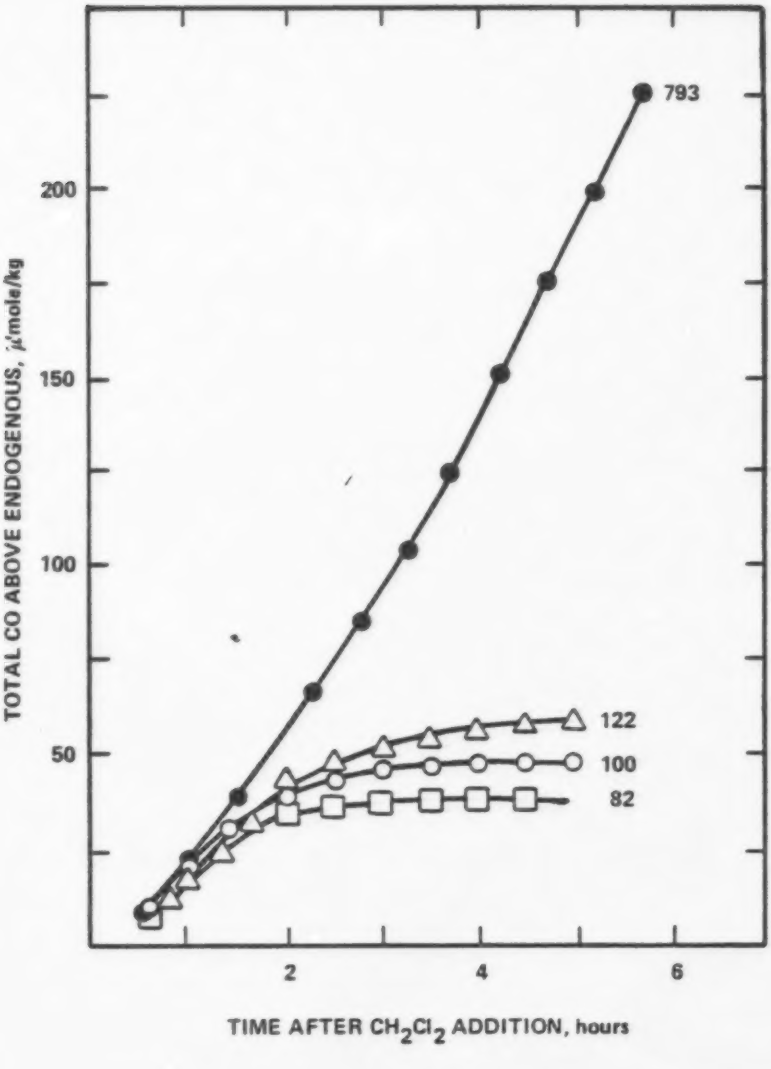

Figure 4-5. Rates of production of CO from DCM given to rats. Each curve represents changes above endogenous CO rate after the dose (in µmoles/kg b. w.) was given by inhalation. From Rodkey and Collison, 1977.

of DCM. Similar results were obtained with germ-free rats obviating intestinal bacteria as a source of CO. In normal rats, DCM in fact inhibited methane production by intestinal bacteria. The same results were also obtained with dibromomethane, dichloromethane, bromochloromethane, and diiodomethane in respective order of magnitude and rate of CO production.

The important finding by Rodkey and Collison (1977) that the metabolism of DCM to CO in rats is rate limited by enzyme saturation to about 25 µmole/hr/kg b.w. explains the seemingly low conversion observed by DiVincenzo and Hamilton (1975) in this same species. Recalculation of their data (Table 4-4) gives a DCM metabolism to CO of about 19 µmole/hr/kg b.w. Comparable results for mice were also obtained by Yesair et al. (1977). These investigators administered by intraperitoneal injection 1.0 and 100 mg/kg ^{14}C-DCM in corn oil. Exhaled ^{14}CO, ^{14}CO$_2$, and unmetabolized ^{14}C-DCM were trapped (^{14}CO after oxidation with Hopcalite® to ^{14}CO$_2$, ^{14}CO$_2$ in aqueous potassium hydroxide, and ^{14}C-DCM on coconut charcoal) and quantified by gas chromatography and radiotracer assay. The 1 mg/kg dose (11.76 µmole/kg) was quantitatively metabolized to CO (0.45 mole/mole DCM) and CO$_2$ (>0.50 mole/mole DCM). The larger dose (11.76 µmole/kg) yielded, in the exhaled air collected for 12 hours, 470 µmole/kg of unmetabolized ^{14}C-DCM (40 percent dose), and 0.20 mole ^{14}CO and 0.25 mole ^{14}CO$_2$. Hence, at least in mice under these experimental conditions, a 12 µmole/kg dose of DCM (1 mg/kg) does not saturate the metabolizing enzymes, whereas 1200 µmole (100 mg) DCM/kg saturates the enzymes and is metabolized at a constant rate of about 20 µmole/hr/kg b.w. The remainder of the dose is excreted unchanged in the exhaled air. Recently McKenna et al. (1979) exposed rats to 50, 500, and 1,500 ppm (174, 1,737, and 5,211 mg/m^3) ^{14}C-DCM for 6 hours and also found the net uptake and metabolism of DCM to CO and CO$_2$ did not increase in proportion to the incremental increase of DCM exposure concentration.

Increasing amounts of unchanged DCM in exhaled air were found with increasing exposure concentration. McKenna et al. (1979) were able to describe the relation of total metabolism of DCM to exposure concentration by nonlinear Michaelis-Menten kinetics. While these estimated rates of DCM metabolism observed in the rodent cannot with absolute certainty be extrapolated to man, there is little reason to doubt that the factors governing the metabolism of DCM in rodents are generally similar in man.

4.2.2 Enzyme Pathways of DCM Metabolism

Figure 4-6 summarizes current knowledge of the enzyme pathways involved in the biotransformation of DCM. The scheme is based on studies *in vivo* and *in vitro* with hepatocytes and microsomal preparations. The preponderance of evidence as noted above from *in vivo* experiments indicates that these enzyme pathways are unique to the dihalomethanes and give rise to both CO and CO_2 in nearly equimolar amounts.

The primary reaction the dihalomethanes undergo appears to be an oxidative dehalogenation first described by Kubic and Anders (1975). These workers found that DCM and other dihalomethanes were metabolized by rat liver microsomal fractions to CO with inorganic halide release. The system required both NADPH and O_2 for maximal activity. These experiments were carried out in a closed vessel, substrates were added without carrier solvent, and CO was determined by the gas chromatographic headspace method. With dibromomethane as the substrate, 3.6 mole bromide was produced per mole CO. In the absence of NADPH, microsomal fractions dehalogenated the methanes without CO formation. Anaerobic conditions substantially reduced the rate of conversion, although some CO formation (20 percent maximal) occurred. Equimolar substrate concentrations of dichloromethane, bromochloromethane, dibromomethane and diiodomethane added to microsomes produced the least amount of CO for dichloromethane, while diiodomethane yielded the greatest amount (7 times DCM). Liver

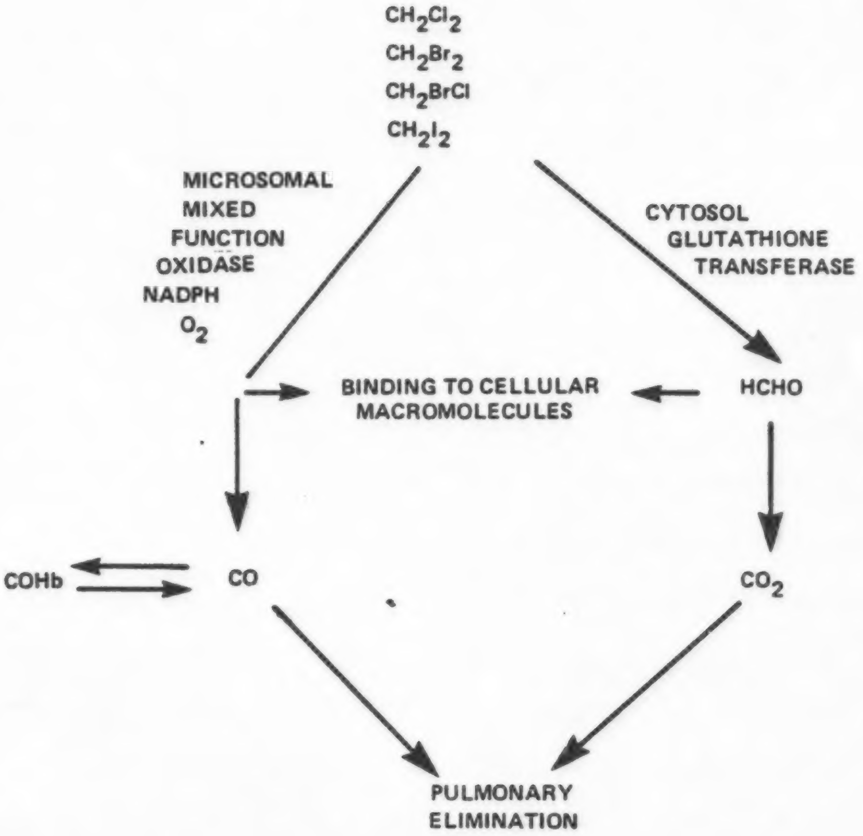
CH_2Cl_2
CH_2Br_2
CH_2BrCl
CH_2I_2
MICROSOMAL MIXED FUNCTION OXIDASE
NADPH
O_2
CYTOSOL GLUTATHIONE TRANSFERASE
BINDING TO CELLULAR MACROMOLECULES
HCHO
COHb
CO
CO_2
PULMONARY ELIMINATION

Figure 4-6. Enzyme pathways of the hepatic biotransformation of dihalomethanes.

microsomes were five times more active than lung and thirty times more active than kidney microsomes. Hogan et al. (1976) also found DCM to be converted to CO by rat liver microsomes requiring aerobic conditions and a NADPH generating system. These workers noted a high correlation between *in vitro* CO production and microsomal cytochrome P_{450} content.

Further evidence of the participation of the P_{450} mixed function oxidase system in the metabolism of dihalomethanes is the observation that dibromomethane and dichloromethane added to microsomal cytochrome P_{450} preparations produce type I binding spectra (Kubic and Anders, 1975; Cox et al., 1976). However, Cox et al. (1976) found that the affinity for P_{450} is less for DCM (K_S, 10 mM) than for chloroform (K_S, 3 mM) or carbon tetrachloride (K_S, 1.5 mM) although carbon tetrachloride and chloroform do not give rise to CO *in vivo* (Miller et al., 1973; Kubic and Anders, 1975; Rodkey and Collison, 1977). Both Kubic and Anders (1975) and Hogan et al. (1976) found that phenobarbital *in vivo* pretreatment induced additional *in vitro* CO production, while cobaltous chloride, which depletes microsomal cytochrome P_{450}, reduced CO microsomal production. Furthermore, SKF 525A, ethylmorphine and hexobarbital (type I substrates) inhibited *in vitro* microsomal conversion of dibromomethane to CO (Kubic and Anders, 1975).

Neither the details of the mechanism of the enzymatic oxidative dechlorination of dihalomethanes to CO, nor the identity of transient intermediates are known. Carbon dioxide, an end product of metabolism *in vivo*, has not been reported to be a product with CO of microsome oxidative dechlorination. Kubic and Anders (1975) found that rat microsomal fractions debrominate dibromomethane in the absence of NADPH, which suggests that this compound may also be converted to one-carbon metabolites other than CO. Van Dyke and coworkers (1970,1971) have described a microsomal P_{450} oxidative dechlorination of chloroethanes and chloropropanes. The system requires NADPH and O_2, and is inducible by phenobarbital administration. Dechlorination occurs optimally in the presence of O_2, although some dechlorination may also occur under anaerobic conditions. Optimal conditions also require inclusion in the system of some unknown (not glutathione) 105,000 g cytosol factor. Unfortunately, the products of the dechlorination reaction were not usually characterized, since the reaction was followed by release of ^{36}Cl. However, in the case of 1,1,2-trichloroethane, the products were identified as mono- and dichloroacetic acid and mono- and dichloroethanol, of which the last compound was the major metabolite.

At least two pathways exist in rat liver for the biotransformation of the dihalomethanes (Figure 4-6). Kuzelova and Vlasak (1966) detected formic acid in the urine of DCM-exposed workers and suggested that DCM was metabolized via formaldehyde to formic acid. Originally Heppel and Porterfield (1948) reported the conversion of dibromomethane and bromochloromethane to stoichiometric amounts of formaldehyde and inorganic halide by a 9,000 g supernatant fraction of rat liver, and also by liver slices and homogenates. The system did not require O_2 but was glutathione dependent. Kubic and Anders (1975) have recently confirmed these findings and localized this metabolic pathway to the cytosol. Ahmed and Anders (1976) extended these findings and showed that the cytosol system is a glutathione transferase found only in the liver which requires no other cofactor than glutathione (cysteine is not a substitute), and is not inducible by phenobarbital or by repeated exposure to DCM or dibromomethane. The substrate order of activity is diiodo > dibromo = bromochloro >dichloromethane, the same order as found for oxidative dehalogenation by Kubic and Anders (1975). It is unlikely that this pathway contributes to CO production via a metabolism of formaldehyde to CO since formaldehyde administration does not produce an increase of COHb in animals or man (Kubic et al., 1974; Rodkey and Collison, 1977; Kasselbart and Angerer, 1974).

The cytosolic glutathione transferase system for dehalogenation of dihalomethanes with the production of formaldehyde appears to be the major source of CO_2 as an end product of the metabolism of DCM in the intact rodent (Rodkey and Collison, 1977; DiVicenzo and Hamilton, 1975; Yesair et al., 1977; McKenna et al., 1979). Neely (1964) has demonstrated that formaldehyde is almost quantitatively metabolized to CO_2. When Neely injected ^{14}C-formaldehyde (^{14}CHO) intraperitoneally into rats at dose levels of 0.25 and 2.5 μmole/kg of body weight, he recovered 82 percent of the dose as CO_2 in exhaled air collected over a 24-hour period. Peak concentrations of $^{14}CO_2$ in exhaled air occurred 1 hour after administration. Neely suggested that the formation of CO_2 occurred from formaldehyde entering the 1-C metabolic pool with transfer to glycine by the folic acid cycle to give serine. Transamination of serine to pyruvate provides entry to the tricarboxylic acid cycle and completes the oxidation to CO_2. In support of this metabolic route, small amounts of ^{14}C-labeled serine and methionine were found in the urine. On the other hand, when ^{14}C-DCM was administered to rats, no ^{14}CHO was detected in the breath, serum or tissues 2 hours later, although substantial changes in tissue formaldehyde content were noted (Rodkey and Collison, 1977; DiVincenzo and Hamilton, 1975).

An additional source of CO_2 production from the metabolism of dihalomethanes is the _in vivo_ oxidation of CO to CO_2. It has long been known that CO is metabolized to CO_2 by various animal tissues (Fenn, 1970). Luomanmaki and Coburn (1969) have also demonstrated $^{14}CO_2$ in the expired air of humans breathing ^{14}CO during a 4-hour period in a closed rebreathing system. Carbon monoxide is metabolized by combining with the reduced form of tissue cytochrome oxidase in the presence of a low O_2 tension, being released as CO_2 (Coburn, 1970). However, the amount of CO_2 generated by oxidation of CO is very small, since the rate of conversion is less than 5 percent endogenous CO from heme catabolism (Tzagoloff and Wharton, 1965). On the other hand, the metabolic rate of conversion of CO to CO_2 appears to increase as a function of body stores of CO and thus blood COHb levels. In dogs, at 10 to 15 percent COHb, the metabolic conversion rate equals the formation of endogenous CO (Luomanmaki and Coburn, 1969). Increased CO production and elevated COHb resulting from catabolism of DCM may stimulate the metabolic production of CO_2 from CO, thus contributing to the total CO_2 produced by DCM metabolism.

Together, microsome oxidative dehalogenation and cytosol glutathione transferase dehalogenation systems (Figure 4-6) account for the CO and CO_2 generated from the metabolism of the dihalomethanes. Since the microsomal system is apparently saturated and rate-limiting at low doses (supra vide), the relative molar amounts of CO and CO_2 produced should provide an index of the activity of the two pathways. However, Yesair et al. (1977) found nearly equal molar amounts of CO and CO_2 with both low and high doses of DCM in mice. Rodkey and Collison (1977) found, for low doses in rats, 1.6 times as much CO produced as CO_2, suggesting greater metabolism by the microsomal oxidative pathway. While it has been observed that equimolar doses of dibromomethane and diiodomethane produce greater COHb levels than DCM produces in rats (Fodor and Roscovanu, 1976; Roth et al., 1975; Rodkey and Collison, 1977), in microsomal preparations (Kubic and Anders, 1975), and in cytosol preparations (Ahmed and Anders, 1976), no information is available on the ratio of $CO:CO_2$ produced by these compounds. The use of isolated hepatocytes may prove useful and avoid many of the difficulties inherent with 'whole animal' experiments.

Cunningham et al. (1979) have used isolated rat hepatocytes to investigate the binding to cellular macromolecules of "reactive" intermediates (possibly formaldehyde) from the metabolism of ^{14}C-DCM. These workers found significant binding of nonextractable ^{14}C to cellular proteins and lipids, although less than that observed with trichloroethylene or carbon tetrachloride. Binding to RNA and DNA was insignificant compared to trichloroethylene. Phenobarbital-stimulated rat hepatocytes showed increased binding of trichloroethylene and carbon tetrachloride metabolites to cell lipids but decreased the binding of DCM metabolites. Reynolds and Yee (1967) studied labeling patterns of ^{14}C-DCM and ^{14}C-formaldehyde in rat liver and found that they have similar patterns. Binding occurred most at the amino acid locus corresponding to serine, and on the acid soluble cell constituents, with smaller amounts in lipid and nucleic acids.

The metabolism of the dihalomethanes by the microsomal oxidative dehalogenation pathway (but apparently not the cytosol pathway) can be modulated by inducers of microsomal mixed-function oxidase system. Pretreatment of animals with phenobarbital was found by some workers to increase blood COHb levels and microsomal production of CO (Kubic et al., 1974; Kubic and Anders, 1975; Hogan et al., 1976), but other investigators found no effect or observed a decrease (Miller et al., 1973; Roth et al., 1975). Roth et al. (1975) suggested that enhanced metabolism may initially be induced by phenobarbital, but the resulting increase in local microsomal levels of CO may be sufficient to inhibit cytochrome P_{450} oxidative dechlorination. Of particular interest to human exposure in the industrial setting is the finding that chronic daily exposures of rats to DCM substantially increased metabolism and COHb blood concentrations, suggesting that this dihalomethane can induce its own metabolism (Kubic et al., 1974; Rodkey and Collison, 1977). Heppel and Porterfield (1948) also reported that repeated administration of bromochloromethane to rats led to an increased rate of dehalogenation. However, Haun et al. (1972) found that continuous exposure of mice to 100 ppm (347 mg/m^3) DCM for 4 to 12 weeks decreased hepatic content of cytochrome P_{450}. Initially, repeated exposures may result in an increase of "reactive" binding of metabolites to cellular components, and in cytochrome inhibition by CO, thus producing cellular functional changes contributing to the hepatotoxicity of DCM.

4.2.3 DCM-Induced Carboxyhemoglobin Formation

Blood COHb accumulates when the amount of endogenous or exogenous derived CO in the body exceeds that of pulmonary elimination. Since Stewart and his associates (1972,1972a) reported the remarkable increase of blood COHb (up to 15 percent from 0.6 percent pre-exposure) in persons acutely exposed by inhalation to DCM vapor, numerous investigations of the phenomenon have been made in experimental animals and man. Studies have been undertaken to determine the dose-response relationship of blood COHb level with DCM air concentration, with duration of exposure, with time-course of COHb blood concentration rise and decline, and with the magnitude of its occurrence in the industrial setting. Because of its metabolism, DCM as a new endogenous source of CO, is additive to exogenous environmental CO as a health hazard. Of particular concern are smokers who maintain significant constant levels of COHb, i.e., 4.6 to 5.2 percent (Stewart et al., 1974; Kahn et al., 1974), and others who may have increased sensitivity to CO toxicity, such as pregnant women and persons with cardiovascular disease. Indeed, Stewart et al. (1972,1972a) have noted that exposure to concentrations of DCM that do not exceed the industrial TLV (200 ppm, 695 mg/m^3) may yield COHb levels exceeding those allowable from exposure to CO itself (35 ppm, 38.5 mg/m^3). Ratney et al. (1974) studied the blood COHb levels (calculated from alveolar concentrations) of a group of young male adults in their workplace where large quantities of DCM were used (a plastic film plant). Workroom air concentrations of DCM averaged 200 ppm (695 mg/m^3)

with no measurable CO. At the beginning of the work day blood COHb levels averaged 4.5 percent. After an 8-hour exposure COHb levels rose to about 9 percent, then declined exponentially to 4.5 percent by the next working day (16 hours later) with an apparent half-time of CO pulmonary elimination of 13 hours. In contrast, 35 ppm (38.5 mg/m^3) CO in the ambient air of nonsmokers produces a blood COHb level at the end of 8 hours of 5 percent or less with a half-time of pulmonary elimination in man of 4 to 5 hours (Peterson and Stewart, 1970; NIOSH, 1972; Lambertsen, 1974).

Stewart and his associates, in a series of studies (Stewart et al., 1972,1972a,1973; Forster et al., 1974; Hake et al., 1974) have shown that blood COHb levels achieved in response to DCM exposure are proportional to the inhaled concentration and to the duration of exposure. Male nonsmokers were exposed to DCM for for 1, 3, and 7.5 hours daily, 5 days weekly. Blood COHb levels were determined for daily pre-exposure and post-exposure times. Their data, which are replotted in part in Figure 4-3, indicate that maximum COHb levels occur with 400 to 500 ppm (1390 to 1737 mg/m^3) exposure and increase with duration of exposure. Similar results were reported in women nonsmokers exposed 1, 3, and 7.5 hours to 250 ppm (869 mg/m^3) DCM for 5 consecutive days (Hake et al., 1974), and (Fodor and Roscovanu, 1976) in male volunteers exposed to 100 and 500 ppm (347 and 1737 mg/m^3) DCM daily for 5 days. In each of these studies, the time course of decay of COHb levels to pre-exposure levels occurred within 24 hours so that a consistent increment in COHb with daily exposure was not observed (Figure 4-7); however, the half-time of COHb disappearance was significantly longer than expected. The longer apparent half-time of pulmonary elimination of CO produced by hepatic metabolism of DCM results from storage of DCM in adipose tissue (*supra vide*), with conversion to CO continuing subsequent to termination of exposure. In these circumstances,

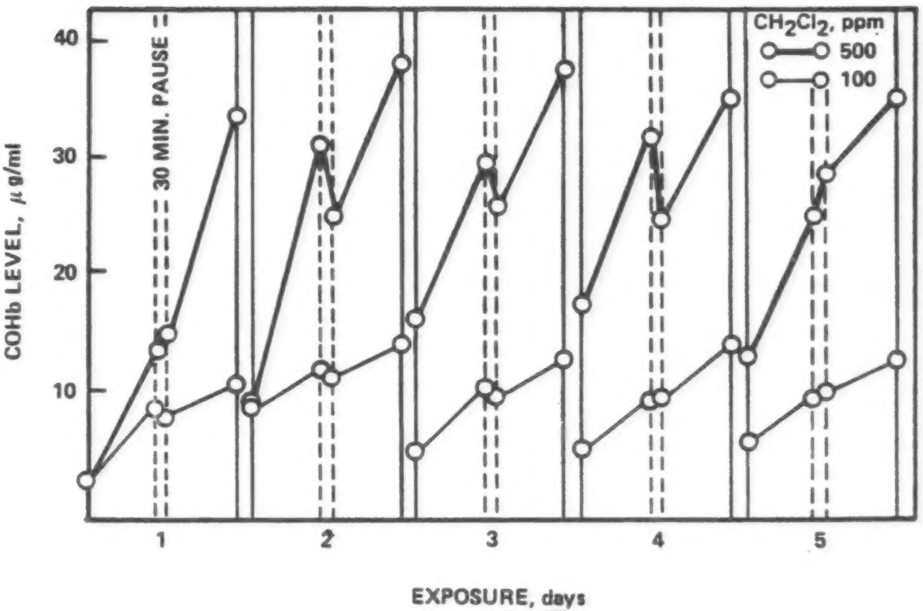

Figure 4-7. Blood COHb level in men during 8 hr exposure for 5 consecutive days to 500 ppm and 100 ppm DCM. COHb percent saturation is equal to μg CO per ml blood divided by 2.5. From Fodor and Roscovanu, 1976.

the biological half-life of COHb derived from DCM commonly observed as 10 to 15 hours is proportional to the body burden of DCM, in contrast to the constant half-life of 4 to 5 hours from CO inhalation (Stewart et al., 1972, 1972a, 1976a, 1976b; Fodor et al., 1973; Ratney et al., 1974; Peterson, 1970).

Physical activity or exercise during exposure to DCM markedly increases pulmonary absorption and body retention (supra vide), but tends to diminish the maximum blood COHb level achieved by the end of the exposure period, although high blood COHb levels are attained 3 to 4 hours after exposure when compared with control sedentary subjects (Stewart and Hake, 1976; Astrand et al., 1975). These findings may be explained by the decrease in half-life of pulmonary CO elimination effected by the increased pulmonary ventilation rate and increased cardiac output with physical activity (Lambertsen, 1974).

The proportionality between DCM inhalation concentration and resultant blood levels of COHb observed in man has been confirmed by animal studies, although with evident species differences. Figure 4-3b shows the relationship between exposure concentration and COHb levels in rats reported by Fodor et al. (1973). For a 3-hour exposure, maximal levels of COHb (12.5 percent) are achieved with about 1,000 ppm (3,474 mg/m^3). Fodor and Roscovanu (1976) noted that a 3-hour exposure with 200 ppm (695 mg/m^3) DCM in man produced COHb levels of about 4.3 percent, but found nearly twice this level in the rat (Figure 4-3b), suggesting that metabolic capacity for CO formation is greater in rats than man. Hogan et al. (1976) found that rats exposed to 440 ppm (1,529 mg/m^3) for 3 hours had maximal COHb levels of about 7 percent, and exposure to 2,300 ppm (7,990 mg/m^3) produced no further increase. Pretreatment of the animals with phenobarbital increased the rate of rise of COHb levels, increased the time the maximum level was maintained, but did not increase the highest level itself. These investigators suggest that endogenous CO inhibition of the P_{450} metabolizing system by CO binding to the cytochrome may occur at very high levels of DCM exposure.

Concentrations of COHb in the blood of rabbits after very short exposures (20 minutes) to DCM inhalation concentrations ranging from 2,000 to 12,000 ppm (6,948 to 41,688 mg/m^3) were found by Roth et al. (1975) to be a linear function of DCM exposure concentration. COHb levels were approximately 5.5 percent at 2,000 ppm (6,948 mg/m^3) and 13 percent at 12,000 ppm (41,688 mg/m^3). With 4 hours of exposure at about 7,000 ppm (24,318 mg/m^3), steady-state blood COHb concentrations of 14 percent were attained. Phenobarbital pretreatment of the rabbits decreased the blood level of COHb achieved with a given DCM exposure, although in parallel experiments phenobarbital stimulated rabbit microsomal benzene hydroxylase and benzphetamine N-demethylase activity. Roth et al. (1975), therefore, also suggested that increased CO production from DCM at the microsome in response to phenobarbital-induced increase of cytochrome P_{450} system inhibits further DCM metabolism.

Haun et al. (1971,1972), in a comparison of dogs and monkeys continuously exposed to 25 and 100 ppm (87 and 347 mg/m^3) DCM for 6 to 13 weeks, found that steady-state levels of blood COHb were maintained throughout the exposure period and were proportional to the exposure concentration. While dogs had higher steady-state blood concentrations of DCM than monkeys at these inhalation concentrations, monkeys had the higher COHb blood levels, suggesting that monkeys have a greater hepatic capacity for CO formation.

Ordinarily, the sole endogenous source of CO and hence COHb is from the physiologic catabolism of heme by the hepatic microsomal heme oxygenase pathway (Coburn, 1973; Tenhunen et al., 1969). The endogenous rate of CO production from this source in normal man is about 20 μmole/hr, producing a blood COHb level of approximately 0.4 percent. There is a close linear correlation between the molar rate of CO production and percent COHb saturation; only 10 to 15 percent of the total body CO is not associated with hemoglobin. Most of this 10 to 15 percent is bound to hemoproteins such as myoglobin and heme cytochromes; about 1 percent is dissolved in body water. The rate of endogenous CO production from heme catabolism is markedly inhibited by exogenous CO sources producing COHb levels of approximately 12 percent saturation, suggesting that blood levels of 12 percent saturation are sufficient to inhibit, by CO binding, hepatic microsomal oxidase systems involved in hemoglobin degradation to CO (Coburn, 1970). The half-life of COHb (4 to 5 hours) arising from heme catabolism or exogenous environmental CO is decreased by increased alveolar ventilation or increased inspired partial pressure of oxygen (Lambertsen, 1974).

To the present time, no studies in man have been reported on either the rate(s) of endogenous formation of CO during DCM exposure or the extent of metabolism of DCM to CO as a percentage of retained dose. It is also not known if DCM is metabolized in man by both the oxidative and nonoxidative enzyme pathways, shown in Figure 4-6, as it is in the rat. Compared with the endogenous rate of CO formation from heme degradation, COHb levels produced by DCM suggest that the rates of CO formation from DCM are in the order of thirty-fold higher than endogenous rates from heme.

Observations in the rat and mouse indicate that their hepatic capacity to metabolize DCM is rate-limited by saturation of the metabolizing enzyme systems at relatively low concentrations of DCM exposure and blood levels (supra vide). A consideration of the data of Stewart et al. (Figure 4-3), relating inhalation exposure concentration of DCM and percent COHb saturation, and the fact that the retained dose of DCM is directly proportional to the level and duration of exposure, shows that the percentage of the retained dose metabolized to CO, and hence COHb, is not constant but decreases at higher doses. Thus, at an exposure of 500 ppm (1,737 mg/m^3) for 7.5 hours in male nonsmokers (Figure 4-3a), the percent COHb saturation and hence metabolism is apparently rate-limited in a manner similar to that in the rodent (Figure 4-3b). Since it can be expected that complete equilibrium exists with inspired air concentration of DCM at 7.5 hours, and for an Ostwald coefficient of 10.5 for blood/air concentrations, then the blood concentration will approximate 0.21 μmole DCM/ml. Assuming that this blood concentration of DCM does indeed saturate hepatic metabolism of DCM, and therefore a zero-order rate of conversion to CO occurs, then as a first approximation a one-compartment open kinetic model with zero-order input can be used to describe the time course of blood COHb as follows:

$$k_o \rightarrow \boxed{V \quad C} \rightarrow k_e$$

where V is the volume of the Hb compartment (90 percent CO is distributed in this compartment (Luomanmaki and Coburn, 1969), k_o, zero order rate of CO formation, k_e, first order rate constant for pulmonary elimination of CO from COHb, and C, the concentration at any time t of COHb formed; then

$$C_t = \frac{k_o}{Vk_e} \left[1-e^{-k_e t}\right]$$

This equation (Wagner, 1975) describes the time course of rising COHb concentration with zero-order formation of CO. With long periods of exposure to DCM, the relation becomes

$$C_t = k_o/Vk_e$$

that is, a steady-state plateau concentration of blood COHb is reached. Using the data of Stewart et al., Figure 4-3, and assuming that a value of 11 percent

COHb saturation corresponds to the steady state concentration for 500 ppm (1,737 mg/m^3) DCM exposure, then for a 70 kg man with a 5 liter blood volume and a normal Hb content a value for k_o of 13 μmole CO/hr/kg can be calculated. This value agrees well with estimates for the rodent (20 to 25 μmole CO/hr/kg) and corresponds to a hepatic clearance of 72 ml/min or about 6 percent of the amount of DCM in the minute blood flow through the liver.

While the accumulation of blood COHb with DCM exposure may be more appropriately described by other first order linear models, and possibly by nonlinear Michaelis-Menton kinetics (McKenna et al., 1979), nonetheless this approximation indicates that the extent of metabolism of an inhaled DCM dose is probably less than 10 percent for a 500 ppm (1,737 mg/m^3) exposure. An adequate kinetic model describing in man the parameters of absorption, distribution, and elimination of DCM, as well as metabolism to CO and COHb, would be of considerable value in evaluating the effects of physical activity, pulmonary and cardiovascular diseases, and concomitant environmental xenobiotic exposures including drugs such as alcohol and barbiturates, of which very little is known. Hake (1979) has developed a computer simulation model, based on the Coburn-Forster-Kane equation, describing the kinetics of CO metabolism using the experimental data of Stewart and his associates. He was able to show that physical activity during exposure to DCM led to a lower blood COHb than the sedentary level, because while increased ventilation increased DCM uptake, it also increased the pulmonary elimination rate constant (k_e) for CO.

4.3 MEASURES OF EXPOSURE AND BODY BURDEN

In the controlled laboratory setting, estimating the DCM absorbed into the body by comparing inspired and alveolar air concentrations, or by measuring blood levels of DCM and then extrapolating these parameters to body dose still remains an imprecise task. The goal, however, is to develop a sufficient data base and knowledge of the kinetics of absorption, disposition, elimination, and metabolism of DCM to enable assessment of the body burden of DCM from acute or chronic exposure in the industrial setting where air concentrations and exposure periods vary widely. Air monitoring, an important control measure, cannot be used as a reliable index of body burden. At present, monitoring levels in blood or alveolar air, and measuring blood COHb levels are the available approaches to estimating body burdens from recent exposures. However, in addition to the lack of reliable pharmacokinetic knowledge necessary to interpret these determinations into accurate and reliable measures of body burden, they are also subject to unknown inter-individual variation from factors such as anthropometric differences, metabolism and work load, age and sex, as well as modifications from drugs and environmental xenobiotics.

Stewart and his associates (1976a) advocate the use of breath analysis to monitor DCM exposure, in part because it is a noninvasive method and avoids the problems associated with multiple blood sampling required for determinations of blood DCM and COHb levels. In addition, breath samples can be readily collected with little inconvenience in the immediate post-exposure period and also at later periods. Analysis of DCM in those samples by infrared spectroscopy or gas liquid chromatography provides both an identification and a measurement of the magnitude of exposure.

Stewart and his associates have constructed a "family" of post-exposure breath decay curves spanning 20 hours from controlled and known inhalation exposures of volunteers in the laboratory. The concentration of DCM in the alveolar air during and after exposure is directly related to the average inhalation exposure concentration. Provided duration of exposure and work intensity are known, the total body burden can be estimated by reference to "standard" breath concentration curves. Because of large variations between individuals exposed under identical conditions, only very approximate DCM body doses can be estimated. The first 2 hours after exposure appear to be the most reliable breath analysis sampling time for estimating the time-weighted average DCM exposure concentration. Stewart et al. (1976a) provide little information on the statistical reliability or the inter-individual variation expected in the predictive values for exposure burdens, although they point out the desirability of constructing "individualized" breath decay curves for every person expected to be exposed to DCM. Recently, Petersen (1978), using the same experimental exposure data base as Stewart et al., has developed empirical equations relating post-exposure breath concentrations with exposure time, duration, and blood COHb levels.

4.4 SUMMARY AND CONCLUSIONS

The kinetics of absorption, distribution and excretion of DCM in man has not been extensively studied; few well-designed, controlled studies are available, particularly for repeated chronic daily exposures to low vapor concentrations relevant to workplace or environmental conditions. Nonetheless, the broad outlines of the pharmacokinetics have been established. Inhalation and lung absorption of DCM vapor in air is the most important and rapid route of absorption into the body. Absorption through the skin by vapor contact or by immersion is slow and does not contribute significantly to body burden. Pulmonary absorption is directly related to the blood/air partition coefficient which, for DCM, is three times greater than for methylchloroform and similar to that of trichloroethylene. Hence, for similar vapor exposure concentrations, pulmonary uptake of DCM will nearly equal trichloroethylene uptake and will be three times greater than methylchloroform uptake. However, as total body dose of DCM is related also to solubility in tissues, at 200 ppm (695 mg/m^3), which was until recently the TLV value for an 8-hr exposure, less than 2 g may be expected to be absorbed into the body of a normal sedentary 70 kg man. The total body dose of DCM increases in direct proportion to inspired air concentration, duration of exposure, and body weight and fat content; it is also increased by physical activity during exposure. Because of its lipid solubility, DCM is distributed widely throughout the body, readily crossing the blood-brain and placental barriers, and concentrating into breast milk. Blood and tissue concentrations achieved during exposure are directly proportional to inspired air concentration and total body dose. DCM has the lowest tissue/blood partition of all structurally related solvents and, for given blood levels, the tissue concentrations for DCM are lower than for other solvents.

After exposure (inhalation, oral, or skin absorption), more than 85 percent of DCM is excreted unchanged via the lungs; less than 2 percent is eliminated in urine, 10 percent or less by metabolism, and the remainder by other routes of elimination. Alveolar air concentration and blood concentration decline in a parallel exponential fashion showing three major components of elimination with half-times of approximately 15, 60, and 360 min. The long half-time of elimination of the adipose tissue compartment (6 hr) together with reports that DCM remains in this compartment 24 hr after exposure indicates that DCM may very slowly accumulate in body fat with chronic daily exposure, particularly in obese persons.

Although less than 10 percent of a body dose of DCM is biotransformed, the metabolism of DCM to CO and CO_2 is unique to and characteristic of dihalomethanes. Biotransformation occurs primarily in the liver but also in the lung and kidney. Metabolism is rate-limited by substrate saturation of metabolizing enzymes and by product (CO) inhibition. In the liver, CO is produced by oxidative dechlorination of DCM by microsomal P_{450} mixed-function oxidase system. A second cytosolic glutathione transferase system dehalogenates DCM to produce formaldehyde which is further oxidized to CO_2. The microsomal system (but not the cytosol system) is inducible by phenobarbital and other microsomal inducers and by DCM itself.

The endogenous production of CO and thence COHb from DCM metabolism is additive to COHb formed from exogenous CO. The formation of COHb from DCM exposures that do not exceed the industrial TLV may yield blood COHb levels exceeding those allowable from exposure to CO itself; hence DCM exposure may result in health hazards and toxicities associated with the solvent itself as well as with CO at the cytochrome level. The kinetics of COHb formation and CO elimination are interdependent with the metabolism of DCM. A functional relationship exists between DCM inhalation concentration and duration of exposure and the time course and peak blood COHb level. The blood COHb level achieved is the result of CO formation from DCM and the kinetics of pulmonary elimination of CO from COHb. Physical activity decreases blood COHb levels by increasing CO pulmonary elimination.

Further research on the disposition and fate of DCM after low chronic vapor exposure is needed and would help in understanding: (1) bioaccumulation in adipose tissue, (2) enzyme mechanisms of biotransformation and intermediate reactive metabolites, (3) kinetics of CO and COHb formation, (4) interactions of DCM and CO metabolism and toxicities with ethanol and other common drugs, and (5) binding to tissue macromolecules.

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5. HEALTH EFFECTS OF DICHLOROMETHANE

5.1 OVERVIEW

The primary results of an acute exposure to dichloromethane (DCM) are central nervous system (CNS) depression, cardiotoxic effects, and increased levels of blood carboxyhemoglobin (COHb), which are a consequence of the metabolic transformation of DCM to carbon monoxide (CO). Hepatic and renal toxicity may result in liver and kidney damage that may or may not be reversible, depending on dose and length of exposure. Ocular toxicity can result in damage to the eye, but complete healing usually would be expected. Few dermal effects have been reported, although dermal exposure may result in more severe systemic effects. DCM may be capable of producing hemolytic anemia in susceptible subpopulations such as those having erythrocytes deficient in glucose-6-phosphate dehydrogenase, although there is little existing evidence.

Fatal and nonfatal acute human effects have occurred upon exposure to DCM. Inhalation exposure or concurrent inhalation and dermal exposures have been the most common. The concentration of DCM in human exposures has usually not been reported. However, the circumstances under which the exposures have occurred suggest that the concentrations have ranged from very high levels (>5,000 ppm, 17.37 g/m^3), in industrial accidents where large volumes of DCM were involved in operations at elevated temperatures, to more moderate levels (100 to 1000 ppm, 347 to 3474 mg/m^3), i.e., those associated with home use of consumer products, specifically paint and varnish remover. Animal studies generally support and confirm the findings noted in cases of acute human exposure. DCM has been found to be mutagenic in bacteria and a mammalian cell line. Extensive information is either not presently available or has not been fully appraised for an assessment of the carcinogenic potential of DCM. The EPA

Carcinogen Assessment Group has deferred a final assessment of the carcinogenicity of DCM until additional data can be thoroughly evaluated.

5.2 HUMAN HEALTH EFFECTS

5.2.1 Overview

CNS effects are related to the narcotic properties of DCM. The onset of these effects is generally rapid, and their persistence is of short duration (normally subsiding within hours after the cessation of exposure). In cases of acute human exposure, these effects have included death, unconsciousness, labored breathing, headache, lassitude, and nausea.

Behavioral and neurological alterations resulting from damage to the nervous system have been reported following acute exposure to DCM. The onset of symptoms ranges from immediate to a latency of several months. The persistence of some of these effects is prolonged, some for at least 20 months after the exposure. The effects include mental depression (which has been known to result in suicide in exposed individuals), personality changes, psychoneurotic reactions, and dysarthria.

The observed cardiotoxic properties of DCM include cardiodepression and cardiosensitization. Human case studies have been reported which included fatalities resulting from, or closely associated with, exposure to DCM, in which myocardial infarction was diagnosed. Nonfatal exposures have caused electrocardiographic (ECG) changes that were similar to those induced by CO. (It is as yet unclear what the relative contributions of DCM and its metabolite, CO, are to those effects.) The case histories of certain exposed individuals suggest the existence of underlying cardiovascular disease. This effect may, therefore, be significant to this human subpopulation.

Hepatotoxicity has not been reported in any human case report, even following fatal exposures. The only evidence of human nephrotoxicity resulting from DCM exposure was a finding of congested kidneys following a fatal exposure.

Ocular toxicity other than eye irritation and congested conjunctivae has not been reported in humans exposed to DCM.

Human studies have reported few hematologic changes as a result of exposure to DCM. *In vitro* studies of human blood from sickle cell patients indicated that DCM could produce hemolysis. Furthermore, it appeared to preferentially lyse sickle cells and other abnormal cell forms. These studies suggest that DCM may produce anemia in predisposed individuals (e.g., those with sickle cell anemia or glucose-6-phosphate dehydrogenase deficiency).

The biotransformation of DCM to CO, and the subsequent formation of COHb, is an important clinical measure of exposure. CO production, resulting from DCM exposure, occurs intracellularly, in close proximity to the biochemical sites of CO toxicity. The CO derived from DCM may therefore be more toxic than that resulting from inhalation of CO, if the usual comparative measure of exposure (COHb levels) is considered. There is no general consensus concerning the relative contributions of the parent compound, DCM, and its metabolite, CO, to the overall toxicity of DCM.

5.2.2 Acute Effects

5.2.2.1 Experimental Exposure--Several experiments have been carried out involving the acute exposure of human subjects to DCM. Fodor and Winneke (1971) and Winneke (1974) used two behavioral tests as indications of CNS function in subjects exposed to DCM: (a) a visual critical flicker frequency (CFF) test to determine the frequency at which intermittent flashes of light appear as a steady or continuous light; and (b) an auditory vigilance test (AVT), which involves the detection of faint and frequently occurring auditory signals.

A study involving female volunteers aged 20-30 years (Fodor and Winneke, 1971) found that when these volunteers were subjected to 4-hr exposures of DCM at concentrations of 300 and 800 ppm (1,042 and 2,779 mg/m^3) (1 week apart), there was a significant decrease in the critical flicker frequency (CFF) of approximately the same degree at both concentrations. The ability to detect sound signals in the AVT test was also reduced by exposure to DCM, especially during the mid-interval of the 4-hour exposure. Winneke (1974) also found that exposure of male subjects to concentrations of 370, 470, and 751 ppm (1,285, 1,633, and 2,609 mg/m^3) for 3 to 4 hours resulted in a decreased CFF, a decreased AVT, and a decreased performance in most psychomotor tasks, especially at 751 ppm (2,609 mg/m^3). These consequences appear to be mediated via direct effects on the CNS, since in the same study 18 subjects exposed to either 50 or 100 ppm (174 or 347 mg/m^3) of the DCM metabolite CO displayed no sign of impaired CNS function.

In a chamber with known dynamic characteristics, Winneke and Fodor (1976) exposed 12 women (ages 22 to 31) to a DCM vapor concentration of 500 ppm (1,740 mg/m^3). Behavioral performance was evaluated by an AVT test and a CFF test to determine cortical alertness. Latency of response and percentage of missed signals were noted for three 1-hour periods composed of a 45 minute vigilance task followed by a 15 minute CFF task. A decreased response in both tests became evident within 30 minutes and the impairments increased progressively through termination of the experiment 60 minutes later. Alveolar and blood DCM concentrations were not measured in these studies, nor were COHb and alveolar CO.

Gamberale et al. (1975) investigated the effect of DCM on psychomotor and cognitive performance in 14 men, ages 20 to 30 years, divided at random into two groups. The first group was initially exposed to progressively increased concentrations of DCM--870, 1,740, 2,600, and 3,470 mg/m^3 (250, 500, 748 and 997 ppm)--through a face mask. Seven days later they were observed under control conditions. The second group was studied under identical conditions, but in reverse order. The subjects were exposed to each concentration level for 30 minutes with no break in exposure; total exposure time for each individual was two hours. At each concentration a peak plateau of alveolar DCM occurred in about 10 minutes. Heart rate showed no change. During the two hours of exposure to DCM, no impairment in any measured performance was observed that was statistically significant, although reaction time appeared to be more irregular during exposure than during control conditions. Numerical ability and short-term memory were unaffected. The experiment did not consider any latency effects.

Putz et al. (1976) exposed six men and six women (ages 18-40 years) to 700 mg/m^3 (201 ppm) of 99.5% pure DCM or to 80 mg/m^3 (7 ppm) CO, for four hours in an 8 m^3 chamber with unspecified dynamics. Alveolar concentrations were measured hourly, and peripheral blood from a finger was taken before and after exposure. Peak alveolar CO after four hours of exposure to DCM was about 60 mg/m^3 (17 ppm) and COHb was 5.1%. Alveolar CO after CO exposure for four hours was 50 mg/m^3 (4 ppm) and COHb was 4.85%. Thus, there was COHb and alveolar CO equivalence between the two compounds. Eye and hand coordination at the end of the exposure period was depressed in subjects exposed to either compound compared with controls, with DCM depression being greater than CO depression. The authors concluded that CO may have been primarily responsible for the decreases in performance after exposure to both compounds.

In one study, Stewart et al. (1972b) observed CNS depression in humans exposed to 3,400 mg/m^3 (977 ppm) 99.5% pure DCM for two hours. However, in a later study (1973), some of these same investigators reported that results from behavioral tests (Romberg equilibrium test, alertness testing, coordination tests, arithmetic tests) were inconclusive in individuals exposed to levels of 170, 350 and 870 mg/m^3 (49, 101, and 250 ppm) DCM for five days per week for up to five weeks.

Stewart and Dodd (1964) examined 11 male subjects from 23-43 years of age, exposed to 741 to 3,431 mg/m^3 (213 to 986 ppm) DCM for 2 hours. Several, but not all, of the subjects exposed to 1,789 mg/m^3 (514 ppm) or higher reported some light-headedness. Impaired CNS function, as represented by measurement of the Visual Evoked Response (VER), or changes in electroencephalograms (EEG) in response to a flashing light, were observed in these same subjects.

Stewart et al. (1972a, 1972b) reported that 1 to 2-hour exposures to concentrations of DCM ranging from 1,740 to 3,480 mg/m^3 (500 to 1,000 ppm) (in one study), and from 741 to 3,431 mg/m^3 (213 to 986 ppm) (in another study), resulted in elevated carboxyhemoglobin (COHb) and alveolar CO values at all dose levels examined. In both reports, the peak COHb levels as well as the amount of COHb remaining 17-24 hours after the DCM exposures were directly proportional to the exposure concentrations.

Stewart and Dodd (1964) studied groups of three to five men and women (25-62 years old) who immersed their thumbs in various chlorinated aliphatic solvents. Within the first 2 minutes of immersion in DCM, all subjects reported an intense burning sensation on the dorsal surface of their thumbs. Within 10 minutes, a feeling of coldness or numbness, alternating with the burning sensation, was reported; the slightest movement of the thumb triggered waves of searing and excruciating pain. Following removal from the solvent, their thumbs were described as "numb and cold" or "asleep." A very mild erythema and a hint of white scaling were the only overt signs of irritation. Within one hour, both the erythema and parathesia subsided.

5.2.2.2 Accidental Exposure--In a case of acute exposure reported by Stewart and Hake (1976), a 66-year-old recently retired executive who refinished furniture as a hobby, worked with a commercial paint and varnish remover containing DCM for 3 hours in his basement. One hour later, he experienced chest pains. Two hours later, he was admitted to a coronary care unit with severe, crushing retrosternal pain. Acute anterior myocardial infarction was diagnosed. The man recovered after an unspecified period of time and returned home. Two weeks after returning home he resumed paint stripping. Again, severe retrosternal pain developed and he was readmitted to a hospital with cardiogenic shock, dysrhythmia, and heart failure. Six months after his second discharge from the hospital, he once again returned to his paint stripping activities. He experienced chest pain and died. No mention of carboxyhemoglobin levels was made in the report.

It is likely that DCM exposure was a factor in the acute myocardial ischemia and death of this man. Although mixed exposure occurred, DCM is strongly implicated as a causative agent by: (1) the high correlation between the cardiovascular effects of pure DCM and a commercially formulated paint stripper (see Aviado et al., 1977, below); (2) the well-established metabolism of this halogenated hydrocarbon solvent to carbon monoxide; (3) the known similar effects of carbon monoxide and DCM on cardiovascular function; and (4) the striking sequence of events that occurred in each episode.

This exposure is considered to be acute because of the close temporal relationship between the exposures and the onset of clinical manifestations and because of the relatively long time between successive exposures. This does not, however, preclude the possibility that cumulative effects occurred.

Kuzelova et al. (1975) reported a fatal case of DCM exposure in a film plate production factory. DCM vapors (concentration unspecified) were sufficient to produce narcosis in a 30-year-old workman who, as a result, fell into a vat containing the solvent. He was unconscious and exhibited acrocyanosis, shivering, labored breathing, and severe chilblains over 40 percent of his body. He suffered repeated anterolateral myocardial infarctions and died 26 hours after the incident.

Another case of accidental human exposure to DCM occurred in a plant where DCM was used to extract oleoresin from dried plant materials (Moskowitz and Shapiro, 1952). Four men were found unconscious after excess DCM vapor apparently escaped from a condensing system. One of the men was dead upon arrival at the hospital. Death was attributed "presumably" to CNS depression. At autopsy DCM was found in tissues, and the kidneys and lungs were congested. The three other men recovered. The only remarkable observation that can be made from the case reports -- which were unfortunately neither detailed nor complete (no blood work was done until the 2nd day of hospitalization) -- is that hemoglobin levels were depressed (76-79% normal values) and red blood cell counts were low (3,550,000 - 3,950,000 mm^3) 2 days after admission. Irritation of eyes, respiratory passages, bronchi, and lungs were also noted.

Studies of human blood *in vitro* from individuals homozygous for sickle cell anemia (Matthews et al., 1977; 1978) have shown that DCM produced hemolysis, preferentially of sickle and other abnormal cell types. These reports suggest that acute exposure to relatively high concentrations of DCM may result in anemia, particularly in individuals who are predisposed to hemolytic anemias, e.g., persons with sickle cell anemia or glucose-6-phosphate dehydrogenase deficiency. However, more compelling evidence is required to ascertain that such an effect exists.

A fatal case of a 13-year-old boy who was removing paint from a bathtub using a paint remover that contained DCM was reported (Bonventre et al, 1977). The victim was discovered dead no more than 9 hours after exposure. Observations at autopsy included congested conjunctivae, cyanosis in the brain, and acute lung congestion and edema. Toluene as well as DCM was detected in the liver, blood, and brain. The concentrations of DCM found in the liver, blood, and brain (14.4 mg/100 g, 51 mg/100 ml, and 24.8 mg/100 g, respectively) indicated to the authors that DCM was the primary agent responsible for death, although they also stated that toluene may have had a contributory and/or concurrent effect. Unfortunately, COHb levels were not reported.

Two individuals were exposed to an unknown concentration of DCM fumes resulting from an accidental leak in a degreasing factory (Benzon et al., 1978). One of them, a 40-year-old male, was found unconscious. When examined 1.5 hours later, he was conscious, although amnesic. His initial arterial blood carboxyhemoglobin (COHb) was 19 percent, decreasing to 11 percent after 20 hours, and to 4 percent after 28 hours. He was discharged from the hospital with no further complications.

The other individual, a 50-year-old man with a history suggestive of ischemic heart disease did not lose consciousness. When examined, his COHb level was 11 percent and an early mid-diastolic heart murmur was found at the lower left sternal border. The ECG showed left anterior fascicular block and sinus bradycardia. The following day the ECG showed bundle branch block and nonspecific ST-T wave changes; at this time the COHb level was 6 percent. The myocardial fraction of creatine phosphokinase (CK-MB) was not elevated, suggesting no myocardial injury. Subsequent ECGs showed no further changes. Thirty-four hours after admission to a hospital, blood levels of COHb had decreased to 3 percent. He was subsequently discharged; no further data were presented. The authors commented that the greatest threat to their patient's health was the continued high levels of COHb, resulting from metabolic conversion of DCM to CO.

Another report of acute toxicity in humans following exposure to DCM concerned a man who worked approximately 4 hours over an open drum of DCM, dipping his bare hands into the liquid to clean copper gaskets (Hughes, 1954). There was no provision for air ventilation in the workroom. Upon exposure, symptoms of excessive fatigue, weakness, sleepiness, lightheadedness, chilly sensations, and nausea were reported. About 2 hours after the exposure, cough and substernal pain developed, and pulmonary edema was diagnosed upon admission to a hospital. DCM was considered to be the pronounced respiratory irritant. In striking contrast to the observations of Stewart and Dodd (1964), no adverse dermal effects were noted in this case.

5.2.3 Chronic Effects

5.2.3.1 Experimental Exposure--Stewart and his colleagues extended their studies of the effects of DCM on human subjects to include longer exposure. In one study (Hake et al., 1974), male volunteers were exposed for 5 days/wk for 5 weeks to DCM concentrations of 50 ppm in week 1, 250 ppm in week 2, 250 ppm in week 3, 100 ppm in week 4, and 500 ppm in week 5 (1 ppm = 3.474 mg/m^3). Three subjects were exposed for 1 hr/day, 3 subjects were exposed for 3 hr/day, and 4 subjects were exposed for 7.5 hr/day. This complex experimental design was further confounded by attempts to separate smoking from nonsmoking populations. The information that can be gathered from this report is summarized in Table 5-1. Alongside is a summary of a companion report from Stewart's group (Forster et al., 1974), which exposed 9 female subjects to 250 ppm DCM for 1, 3, or 6.5 hr/day for 5 days.

TABLE 5-1. COHb CONCENTRATIONS IN NON-SMOKERS EXPOSED TO DCM AT 250 ppm (869 mg/m^3) FOR 5 DAYS

Exposure Time hr/day	Mean of Daily Maximum Observed Average COHb (%)	
	Male (n)[a]	Female (n)[b]
Pre-exposure	0.9 (5)	1.4 (8)
1	3.3 (1)	3.5 (3)
3	7.0 (1)	5.1 (1)
7.5	9.6 (3)	10.1 (4)

[a] Hake et al., 1974 [b] Forster et al., 1974

Carboxyhemoglobin levels up to 10 percent were reported in 10 men (20 to 39 years old) and 9 women (20 to 41 years old) exposed to DCM concentrations between 40 and 500 ppm (139 and 1,737 mg/m^3) by inhalation (Peterson, 1978). Exposure durations were between 1 hour and 7.5 hours per day for not more than 5 successive days.

5.2.3.2 Accidental Exposure--Degenerative nervous system disorder was reported in a 39-year-old chemist occupationally exposed for about 5 years to air concentrations of pure DCM estimated to range from 660 to 900 ppm (2,293 to 3,127 mg/m^3) (Weiss, 1967). No liver or kidney damage or ECG alterations were reported. Dermal contact had also occurred and erythema and fissures appeared on the hands and forearms. This individual's progressive visual and auditory illusions and hallucinations were correlated with exposure to DCM. Neurologic and psychiatric examinations excluded an underlying psychosis. Toxic encephalosis due to occupational exposure to DCM was diagnosed.

Collier (1936) reported four cases of occupational exposure to paint remover containing approximately 96 percent DCM. The men, all of whom were professional painters, had been exposed to lead for 5-14 years. During one autumn, while they were engaged in removing paint, the workers complained of loss of appetite, dullness, faintness, and giddiness, while using the remover and during the following few hours. Two of the workers were examined by the author and the findings were reported in detail. One painter, aged 42, complained of leg and arm pains, precordial pain, great fatigue, and blurred vision. The author diagnosed the symptoms as slight chronic lead intoxication and acute dichloromethane-induced toxemia, since the acute symptoms subsided upon cessation of working with the paint remover. The second painter, aged 45, experienced tingling of the hands and feet, in addition to symptoms of fatigue and drowsiness. Upon cessation of exposure to DCM, all of the symptoms subsided.

Barrowcliff (1978) and Barrowcliff and Knell (1979) reported that an individual exposed to 300 to 1,000 ppm (1,042 to 3,474 mg/m^3) DCM for 3 years developed bilateral temporal lobe degeneration. This was thought to result from chronic CO intoxication as a result of exposure to DCM.

There is one epidemiological study of workers exposed to DCM. In this study, Friedlander et al. (1978) compared the mortality experience of 751 employees exposed to DCM with industrial workers not exposed to DCM and with New York State male populations. The authors asserted that DCM exposure had no adverse effect on mortality. While the data support this conclusion, there are certain limitations in the experimental design that may limit the validity of the findings: (a) use of a broad definition of exposure, which may diminish some of the differences between internal ("industrial") controls and the exposed population; (b) use of subject groups that permitted the "healthy worker" phenomenon to influence comparisons; and (c) use of small sample sizes in specific mortality categories, despite a rather large total population.

5.2.3.3 Epidemiology--Friedlander et al. (1978) conducted an epidemiological study of Eastman Kodak Company (Rochester N.Y.) male workers exposed continuously to 104 to 4,176 mg/m^3 (30 to 1,200 ppm) DCM for up to 30 years. No increase was found in the incidence of cancer-related deaths when compared with three control groups consisting of other Kodak employees, and subjects taken from New York State and United States male mortality tables.

5.2.4 Relationship of CO and COHb to DCM Toxicity

It was first observed by Stewart et al. (1972b) that inhalation of DCM is followed by a sustained elevation of COHb concentration. Several studies demonstrate a direct relationship between peak levels of COHb formation and both length of exposure to and concentration of DCM (Hake et al., 1974; Forster et al., 1974).

Investigations of the source of the CO induced by DCM exposure have centered around two possible explanations. The first of these was proposed by Settle (1975), who suggested that the rise of COHb levels seen with DCM exposure was due to a change in the affinity of Hb for CO induced by DCM. Several subsequent reports have indicated that this is not the case but rather that DCM is biotransformed into CO (Dill et al., 1978; Collison et al., 1977). It was further demonstrated that DCM had no effect on the kinetics of the oxyhemoglobin dissociation curve (ODC), and that any shift of the ODC to the left was due solely to CO formation from DCM metabolism. It also appears that the pulmonary loss of CO formed from DCM exposure is slower than when subjects are directly exposed to CO (Ratney et al., 1974).

According to case studies reported by Stewart and Hake (1976), the use of a paint and varnish remover for 3 hours produced a level of about 9 percent

COHb (4 hours after removal from exposure). Elevation of COHb level to saturations greater than 5 percent can adversely affect a patient with angina pectoris or other cardiovascular diseases (Aronow and Isbell, 1973; Anderson et al., 1973; Scharf et al., 1974). Stewart and Hake (1976) state further

> ". . . because it is so sustained following exposure, the cardiovascular stress produced by elevated COHb levels, derived from CH_2Cl_2 metabolism, is greater than that resulting from equally high COHb levels derived from CO."

and:

> "The COHb resulting from the metabolism of DCM is additive to the COHb level attained from exposure to other exogenous sources of CO. For example, a paint remover exposure that results in a 10 percent COHb saturation level when added to a heavy smoker's pre-existing COHb level of 10 percent will produce headache and nausea in the healthy, and sufficient cardiovascular stress in the patient with coronary heart disease to be dangerous."

A comprehensive discussion of CO effects on the CNS and cardiovascular system is also presented in the EPA Document, "Air Quality Criteria for Carbon Monoxide" (Environmental Criteria and Assessment Office, 1979).

5.3 EFFECTS ON ANIMALS

5.3.1 Overview

Animal studies generally support and confirm the findings noted in cases of human exposure to DCM. This is true of the depressant effects of DCM on the central nervous system. The primary cardiovascular effect of DCM has been shown by animal studies to be decreased myocardial contractility. DCM is also capable of sensitizing the heart to epinephrine-induced arrhythmias.

Animal studies have reported few hepatic changes, even at doses ranging from the LD_{50} to "near lethal" doses. Some species differences have occurred (dogs were more susceptible than mice), but the liver changes, nonetheless, have been generally minor.

The number of studies describing renal changes was small. In contrast to hepatotoxicity, mice were more susceptible to nephrotoxicity than dogs, but again, the changes reported were minimal.

Two reports, both from the same laboratory, suggested that DCM may affect pancreatic function.

In rabbits, moderate to severe conjunctival changes occurred following instillation of DCM, and corneal thickness and intraocular tension increased. These changes persisted for 1-2 weeks.

Animal studies reported minimal hematologic changes. However, one subchronic study using dogs noted a variety of changes that suggested DCM exposure could result in hemolytic anemia.

5.3.2 Acute Effects

Studies investigating mortality following short-term exposure to DCM are summarized in Table 5-2. Good agreement between doses causing the reported effects is seen within each route of administration category with the exception of one intraperitoneal value reported by Zakhari (1977). The oral LD_{50} data are also similar to intraperitoneal LD_{50} data. Oral and intraperitoneal data cannot be compared with inhalation data due to the "first pass effect" which occurs following oral or intraperitoneal administration.

Morbidity resulting after short-term exposure includes effects on several different organs. Ocular damage, liver damage, kidney damage, changes in cardiac parameters and increased pancreatic bile duct flow all occur.

5.3.2.1 Central Nervous System Effects--A NIOSH report (1976) records that Berger and Fodor (1968) exposed rats to very high DCM concentrations ranging from 2,800 to 28,000 ppm (9.7 to 97.27 g/m^3). In these exposures an initial period of excitation was followed by a deep narcosis accompanied by a decrease in muscle tone and a reduction in electroencephalogram (EEG) activity. Rats

TABLE 5-2. ACUTE LETHAL TOXICITY OF DCM

Route of Administration	Species	Dose	Duration of Exposure	Effect	Reference
Oral	mice	1987 mg/kg	-	LD_{50}	Zakhari (1977)
	rats	4368 mg/kg	-	LD_{50}	Ugazio et al. (1973)
	rats	2388 mg/kg	-	LD_{50}	Kimura et al. (1971)
Intraperitoneal	mice	448 mg/kg	-	LD_{50}	Zakhari (1977)
	mice	1330 mg/kg	-	100% survival	Plaa and Larson (1965)
	mice	1995 mg/kg	-	20% survival	Plaa and Larson (1965)
	mice	1990 mg/kg	-	LD_{50}	Klaassen and Plaa (1966)
	mice	1900 mg/kg	-	LD_{50}	Gradiski et al. (1974)
	dogs	1260 mg/kg	-	LD_{50}	Klaassen and Plaa (1967)
Inhalation	mice	14,100 ppm	6 hours	LD_{50}	Gradiski et al. (1974)
	mice	16,100 ppm ppm	7 hours	LD_{50}	Svirbely et al. (1947)
	rats	25,600-28,000 ppm	1.5 hours	(a)	Berger and Fodor (1968)
	rats	16,100-18,150 ppm	6 hours	(a)	Berger and Fodor (1968)
	guinea pigs	11,550 ppm	6 hours	LD_{50}	Balmer et al. (1976)

(a) Cessation of brain electrical activity in all animals. 1 ppm = 3.474 mg/m^3

exposed to concentrations of 25,000 to 28,000 ppm (86.9 to 97.27 g/m^3) ceased to exhibit electrical activity after only 1.5 hr; those exposed to concentrations between 16,000 and 18,000 ppm (55.58 and 62.53 g/m^3) ceased electrical activity after 6 hr. Lower concentrations 5,000 to 9,000 ppm (17.37 to 31.27 g/m^3) produced long sleeping periods without the frequent desynchronization phases characteristic of normal sleep. Sleep behavior was even altered at the lowest dose examined 2,800 ppm (9,727 mg/m^3), where a 14-hr exposure resulted in abnormal rapid eye movement (REM) sleep. Other studies have also utilized sleep behavior as an index of CNS function. Fodor and Winneke (1971) have demonstrated in the rat a linear dose-response relationship between REM sleep patterns and 24-hr DCM exposures. They found that total sleep time and time between two REM periods increased with increasing DCM concentrations of 500, 1,000, and 3,000 ppm (1,737, 3,474, and 10,422 mg/m^3). A concentration of 5,000 ppm (17,370 mg/m^3) administered for 1 hr to male rats was sufficient to demonstrate a decrease in running activity (Heppel and Neal, 1944). Running activity increased after exposure, but was lower than activity in the same male rats when they had not been exposed to DCM. Similarly, Thomas et al. (1971) reported that the spontaneous activity of mice was decreased by a 3-hr exposure to a 1,000 ppm (3,474 mg/m^3) concentration of DCM.

5.3.2.2 Carbon Monoxide Formation and Cardiovascular Effects--Much of the definitive work concerning the formation of COHb as a result of DCM exposure and metabolism has been performed in animal model systems.

In 1973, Fodor et al. examined COHb levels in the rat after a 3-hour exposure to DCM at a concentration of 100 ppm (347 mg/m^3). The COHb level after treatment was estimated at 10.9 percent vs 0.4 percent as the control COHb value. Blood COHb levels in rabbits exposed for 4 hours to DCM levels of between 2,000 and 12,000 ppm (6.9 and 41.7 g/m^3) were found to be a linear function of DCM concentration, approximately 5.5 percent at 2,000 ppm (6.9 g/m^3) and 13 percent at 12,000 ppm (41.7 g/m^3) (Roth et al., 1973).

Several investigators have demonstrated with radiolabelled DCM that CO formed during exposure to DCM is a metabolite of the parent compound (Roth et al., 1973; Carlsson and Hultengren, 1975; MacEwen and Vernot, 1971). DiVincenzo and Hamilton (1975) administered radiolabelled DCM to rats intraperitoneally (i.p.); 24 hours later it was found that 91.5 percent of the DCM was eliminated unchanged in the expired air and only 2 percent was eliminated as CO. Hogan et al. (1976) have determined that the mixed function oxygenase system of hepatic microsomes is responsible for the metabolic conversion of DCM to CO. They have also suggested that the apparent non-inducibility of CO formation may be due to endogenous CO inhibition of the metabolizing enzyme system.

Aviado et al. (1977) used pentobarbital-anesthetized, artificially ventilated, open-chested dogs to examine the cardiovascular effects of exposure to 0.5, 1.0, 2.5, and 5 percent pure DCM (5,000, 10,000, 25,000, and 50,000 ppm) (1 ppm = 3.474 mg/m^3), as well as to the same concentrations of a paint stripper composed of 90.2 percent DCM, 4.2 percent methanol, 3.2 percent isopropanol, and 2.4 percent toluene. Pure DCM or paint stripper was administered via an endotracheal catheter for a period of 5 minutes. At the higher doses (2.5 percent and 5 percent), all hemodynamic effects were consistent with primary depression of myocardial contractility (Table 5-3): left ventricular (LV) dp/dt (the time-dependent rate of rise of ventricular pressure which is a measure of myocardial contractility), fell, as did LV pressure. Left ventricular end-diastolic pressure (LV filling pressure) rose. However, cardiac output fell. There was a fall in mean arterial pressure; yet, calculated peripheral vascular resistance (MAP-central venous pressure/cardiac output) rose. Heart rate fell nonsignificantly when 5 percent DCM (50,000 ppm) (174 g/m^3) was employed.

TABLE 5-3. SUMMARY OF CARDIOTOXIC ACTION OF 5% DICHLOROMETHANE

Function[a]	Taylor et al. (1976)	Aviado et al. (1977)[b]
HR	1% ↑ (NS)	11% ↓ (NS)
MAP	0%	4% ↓ (NS)
LVP	0%	4% ↓ (NS)
CVP	0%	---
LVEDP	9% ↑ (NS)	125% ↑
LVdP/dt	17% ↓	22% ↓ (NS)
CO	14% ↓	36% ↓
SV	14% ↓	33% ↓
VR	25% ↑	44% ↑
Comments:	Rabbits	Dogs
	Pentobarbital-anethestized Spontaneously breathing Closed-chested 1-min. exposure (except 1.5 min. for CO) Static dose (5%)	Pentobarbital-anesthetized Artificially ventilated Open-chested 5-min. exposure Increasing doses (0.5, 1.0, 2.5, and 5.0%)

[a] Key:
HR - Heart Rate
MAP - Mean Arterial Pressure
LVP - Left Ventricular Pressure
CVP - Central Venous Pressure
LVEDP - Left Ventricular End-Diastolic Pressure
LVdP/dt - Left Ventricular Rate of Time-Dependent Pressure Change
CO - Cardiac Output
SV - Stroke Volume
VR - Vascular Resistance (Peripheral or Systemic)
RR - Spontaneous Respiration Rate
MV - Minute Volume
NS - Not significantly different from control values at $p < 0.05$

[b] Results presented are for the highest dose (5%) of dichloromethane

The authors concluded that the effects were compound-related and that DCM exerted a negative inotropic action.

The effects of DCM on cardiovascular function were studied in groups of six male New Zealand white rabbits (Taylor et al., 1976). The animals (2.4-3.4 kg b.w.) were anesthetized with pentobarbital and bilaterally vagotomized; results were recorded for 1-minute exposures to 5 percent (50,000 ppm, 174 g/m^3) DCM in air (except for cardiac output measurements which were over a period of 1.5 min.), enriched to 40 percent oxygen and delivered via an endotracheal tube to spontaneously breathing animals. No changes were observed in the mean arterial pressure, left ventricular pressure, left ventricular end-diastolic pressure, or heart rate. However, significant depression of left ventricular dP/dt (rate of rise in ventricular pressure, an index of myocardial contractility), cardiac output, and stroke volume occurred. Exposure to DCM resulted in increased peripheral vascular resistance, apparently to compensate for the decreased cardiac output and to maintain arterial pressure (Table 5-3).

Aviado's study in dogs correlates well with Taylor's findings in rabbits. At 5 percent, DCM decreased left ventricular dP/dt, cardiac output, and stroke volume. An increase in left ventricular end-diastolic pressure and peripheral vascular resistance was observed in both animal models. The mean arterial pressure and left ventricular pressure were not affected in either study.

One difference between the studies of Aviado and Taylor was the lack of change in the heart rate of rabbits compared with an apparent decrease in dogs. This could be due to a difference in the length of exposure, since rabbits were exposed for only 1 minute, and dogs were exposed for 5 minutes. Also, vagotomy of the rabbits may have prevented a parasympathetically-mediated fall in heart rate. The only other difference between the two studies was that left ventricular end-diastolic pressure did not change in rabbits, but significantly increased in dogs.

Trained male beagle dogs (size and sex unspecified) were repeatedly exposed to 37,000 and 70,000 mg/m^3 (10,650 and 20,150 ppm) pure DCM (Reinhardt et al., 1973). After 5 minutes of exposure to DCM, 0.008 mg/kg epinephrine was injected intravenously. Sensitization to epinephrine did not develop. Higher concentrations of DCM could not be given because the animals became hyperactive, similar to Stage II general anesthesia.

Aviado and Belej (1974) exposed Swiss mice (25-35 g) five times to DCM concentrations of 20 and 40 percent v/v in oxygen. A face mask of (unspecified) description was used. Cardiac arrhythmia was produced by the higher concentration. Intravenous epinephrine 0.006 mg/kg sensitized the heart to arrhythmias induced by the lower dose.

Loyke (1973) induced chronic renal hypertension in 13 Sprague-Dawley rats (100 g, sex not specified). Both poles of one kidney were ligated and the contralateral one was removed. After maintenance of high systolic pressure for three months, 11 experimental rats and three controls were injected subcutaneously with 2 mg/kg unspecified DCM, biweekly for 15 doses. Two hypertensive rats were used for positive controls and 11 normotensive rats as naive controls. DCM reduced the systolic blood pressure of the hypertensive rats from 200 to 160 mm Hg. The positive controls remained hypertensive. DCM was ineffective in reducing blood pressure in normotensive rats. No changes ascribable to DCM were seen in the liver.

Douglas et al. (1976) and Wilkinson et al. (1977) used spontaneously hypertensive rats weighing 400 mg (sexes not specified). Six were injected subcutaneously with 90 mg/kg unspecified DCM. Six others were used as controls. DCM reduced the blood pressure of the hypertensive rats by about 10 percent but was ineffective in reducing blood pressure of the normotensive rats.

Adams and Erickson (1976) exposed eight trained mongrel dogs (size and sex not specified) to 1,700, 3,400, 6,800 and 17,400 mg/m^3 (489, 979, 1,957 and 5,009 ppm) DCM for two hours via a permanent tracheotomy. The animals were exposed repeatedly but randomly to all concentrations. Frequency of exposure was not given. COHb, which continued to increase for two hours after exposure, was both time- and dose-responsive. The slopes of the increase showed that the higher blood levels increased faster at higher concentrations than at lower concentrations. Cardiovascular effects resembled those induced by epinephrine, except that the heart rate did not change. DCM increased blood pressure, increased coronary flow, increased inotropic action and induced arrhythmias.

Pryor et al. (1978) reported an experiment with an open-chest mongrel dog (size and sex unspecified). The blood concentration of DCM after 1.5 minutes of exposure to vaporized DCM of unspecified concentration with oxygen supplementation was 2,320 μg/ml and without oxygen supplementation was 2,680 μg/ml. A second exposure to DCM was given one hour after the first. After both exposures, heart rate slowed, blood pressure fell and aortic blood flow decreased. During recovery after the first exposure, tachycardia and hypertension developed. After the second exposure, the animal died.

Differences in the above studies in terms of the effects of DCM on blood pressure, inotropic action, and induction of arrhythmias may be due to the use of different species of animals and different doses of DCM.

5.3.2.3 Effects on the Eye--Ballantyne et al. (1976) studied the effects of liquid DCM on various ocular parameters by instilling 0.1 and 0.01 ml of DCM in rabbits' eyes. Lachrymation persisted for a week, inflammation of the lids and conjuctivae for two weeks, conjunctival edema for a week, sloughing for three days, and increased corneal thickness for nine days. Iritis and keratitis appeared within six hours and lasted for seven and fourteen days, respectively. Intraocular pressures increased also. Increased corneal thickness developed in rabbits exposed to DCM vapor at concentrations of 1,750 and 17,500 mg/m^3 (504 and 5,040 ppm).

5.3.2.4 Effects on Internal Organs and Metabolism--Harms et al. (1976) cannulated the bile duct of male Sprague-Dawley rats (350-450 g) after pretreatment with intraperitoneal injections of 670 mg/kg DCM dissolved in corn oil. ^3H-Insulin was instilled into the duct 24 hours later. DCM seemed to induce an increase in pancreatic bile duct flow which was unrelated to any observed hepatic effect. Hamada and Peterson (1977), in a follow-up study, intraperitoneally injected 860 mg/kg DCM (dissolved in corn oil) in male Sprague-Dawley rats (280-320 g). The bile duct was cannulated and bile duct pancreatic flow and its contents were measured and compared with controls. DCM induced increased pancreatic bile duct flow, decreased protein concentration, and increased chloride, sodium and potassium. Bicarbonate was unaffected. There was no statistically significant difference in wet weight of the pancreas or in total bile flow; this may indicate a reduced hepatic bile flow. An experiment with secretin indicated that these changes were not related to that substance or, after an ancillary experiment with atropine, to any cholinergic effect.

Plaa and Larson (1965) injected 10 male Swiss mice (18-30 g) intraperitoneally with 1330 mg/kg of DCM (source and quality unidentified) dissolved in corn oil. No glycosuria or proteinuria was detected 24 hours later. Two surviving mice of ten that had been injected with 1,995 mg/kg DCM had proteinuria but not glycosuria. No renal histopathology was seen after the lower dose. Proteinuria following the higher dose suggested that there might have been some tubular damage.

Klaassen and Plaa (1966) also injected male Swiss-Webster mice (25-35 g) intraperitoneally with 13.3 g/kg analytical grade DCM. These mice showed no glycosuria, proteinuria or changes in Bromsulphalein (BSP)® excretion 24 hours after injection. Two groups of ten mice each were gavaged for three days with 5 g/kg 60 percent ethanol and equicaloric solutions of dextrose. All animals were then injected with DCM, and urine was collected and analyzed 24 hours later. BSP excretion remained within control limits, indicating the absence of a renal lesion, but the authors reported "a few kidneys exhibited hydropic degeneration with minimal necrosis of the convoluted tubules."

Klaassen and Plaa (1966) injected Swiss-Webster mice (25 to 35 g) intraperitoneally with analytical grade DCM. Doses of 13,300 mg/kg had no effect on BSP retention or on SGPT activity 24 hours after injection. No histopathology was seen upon examination of the liver.

In the dog, however, effects on the liver by DCM were reported by Klaassen and Plaa (1967). They derived an ED_{50} for SGPT elevation of 798 mg/kg (the LD_{50} was 1,260 mg/kg). Histopathology showed moderate neutrophilic infiltration in the sinusoids and portal areas. Necrosis was not seen. At "near lethal" doses there was vacuolization of centrilobular hepatocytes.

Reynolds and Yee (1967) gavaged fasted male Charles River rats (100 to 300 g) with doses of ^{14}C-DCM up to 2,210 mg/kg, dissolved in mineral oil. The rats were sacrificed 24 hours later. No liver necrosis was seen and there was no change in glucose-6-phosphatase activity. Labelled DCM (^{14}C) was found in hepatic lipids and proteins, minimally in the lipids and in high concentrations in proteins. Similar patterns of incorporation were found in liver microsomes.

In contrast to Reynolds and Yee's findings of minimal lipid incorporation of ^{14}C after gavage, Bergman (1978) found that after 10 minutes of inhalation of ^{14}C-labelled DCM by mice (strain, sex, weight, unspecified; DCM unspecified) whole body autoradiography showed DCM to have ". . . a strong fat affinity". Metabolites appeared immediately in the bronchi, the liver and the kidney and persisted for as long as 48 hours. The formation of CO also produced radioactivity in blood and protein. Differences between the two studies may have been due to differences in animals, route of administration and dose.

Weinstein et al. (1972) exposed female ICR mice (13 to 20 per group, 23 to 27 g) continuously to approximately 17,000 mg/m^3 (4,893 ppm) vaporized technical grade DCM in Thomas Domes, at pressures slightly below ambient. Temperature, humidity, CO_2 and air flow were monitored and controlled. During a 24-hour period, there was a progressive decrease of spontaneous activity. Body weight decreased, liver weight increased absolutely and as a ratio to body weight, liver triglycerides increased (indicating liver toxicity), glycogen decreased, and protein synthesis was reduced as shown by reduction of incorporation of ^{3}H-leucine.

Many histological changes appeared. Fatty infiltration after 24 hours involved the entire lobule; centrilobular hepatocyte nuclei became smaller and dense, and ballooning developed. Staining showed glycogen reduction. Progressive changes began after 12 hours of exposure. Smooth (SER) and rough (RER) endoplasmic reticulum showed changes: polysomes broke down, ribosomal particles detached from RER in the centrilobular cells, RER membranes broke up into vesicle, pernuclear cisternae dilated, and lipid droplets increased. Mitochondria, however, were unaffected. The authors concluded that the pattern of liver damage was similar to that following carbon tetrachloride exposure.

Morris et al. (1979) exposed male Hartley guinea pigs weighing 500 to 750 g for six hours to an 18,000 mg/m^3 (5,181 ppm) vapor concentration of reagent grade DCM. Animals were sacrificed immediately after exposure and liver samples were taken. Liver and serum were analyzed for triglycerides which increased markedly in the former and decreased in the latter. Liver phospholipids showed no change nor did serum-free fatty acid. Liver slices were incubated with ^{14}C-palmitic acid. The uptake of ^{14}C was similar to controls. Uptake after ^{14}C-leucine did not differ between controls and exposed samples.

Differences in the above two studies in protein synthesis may be due to different periods of exposure and the use of different animal species.

5.3.3 Chronic Effects

5.3.3.1 Central Nervous System Effects--Few studies have examined the effect of chronic DCM exposure on CNS function. One assumes the reason for this to be that chronic DCM exposure would elicit many of the same types of responses produced in acute studies. This appears to be borne out by the few chronic studies that have looked at CNS function. For example, Heppel et al. (1944) exposed dogs, monkeys, rabbits, guinea pigs, and rats to DCM at a concentration of 34,800 mg/m^3 (10,000 ppm), 4 hr/day, 5 days/wk for 8 weeks. They found that all animals became inactive during each exposure, but some went through an initial excitement stage, very similar to the findings in the acute study of Berger and Fodor (1968). Weinstein et al. (1972) had also reported decreased activity in female mice at the 24-hour juncture of a 7-day, continuous study of 17,400 mg/m^3 (5,000 ppm) of DCM. Along with the lethargy, the mice were also observed to assume a hunched posture and to develop yellow, greasy, rough-appearing hair coats. After 96 hours of exposure, normal activity, for the most part, resumed. At 168 hours of exposure, the only abnormal sign that remained was the appearance of emaciation and dehydration. Thomas et al. (1972) reported a paradoxical increase in the spontaneous activity of female mice that had been continuously exposed to DCM at 87 mg/m^3 (25 ppm) for 14 weeks. Raising the exposure concentration of 348 mg/m^3 (100 ppm) resulted in a response no different from control. Raising the exposure level to 3,480 mg/m^3 (1,000 ppm) for a continuous 4-week study resulted in a decrease in observed spontaneous activity (Heppel and Neal, 1944).

5.3.3.2 Effects on COHb Levels--Only one study in the literature has looked at the effects of chronic low level concentrations of DCM upon COHb formation. Haun et al. (1972) reported significantly elevated COHb levels in dogs and monkeys exposed to 25 and 100 ppm (87 and 347 mg/m^3) DCM. The 100 ppm monkeys showed this highest COHb percentage, followed by the 100 ppm dogs, then the 25 ppm monkeys. The 25 ppm dogs did not have significantly elevated COHb levels. Additionally, the authors reported finding no significant macro-or microscopic changes in any organ in either monkeys or dogs after up to 100 days of chronic, low level (25 ppm and 100 ppm) DCM exposure.

5.3.3.3 Effects on Internal Organs and Metabolism--Many of the "high-dose" chronic animal studies with DCM have revealed a certain degree of liver and kidney involvement as target organs. The magnitude of this involvement increases in such a way that extremely high doses of DCM (depending upon dosage and duration of exposure) can produce toxic effects upon these organs. Heppel et al. (1944) have observed moderate centrilobar congestion and fatty degeneration of the liver in dogs and guinea pigs exposed to 10,000 ppm (34,740 mg/m^3), 4 hr/day, 5 days/wk for 8 weeks. Weinstein et al. (1972) have reported identical findings after exposing mice to 5,000 ppm (17,370 mg/m^3) continuously for 7 days. High mortality was observed in the studies by MacEwen et al. (1972) where 14 weeks of continuous exposures at 1,000 or 5,000 ppm (3,480 or 17,400 mg/m^3) resulted in severe toxic effects and a high degree of mortality in mice, rats, dogs, and monkeys.

More importantly, chronic mouse studies by Weinstein and Diamond (1972) and Haun et al. (1972) have revealed that continuous exposure to even such low levels of DCM as 100 ppm (347 mg/m^3) can effect changes in both liver function and cell architecture.

Savolainen et al. (1977) exposed male rats to 489 ppm (1700 mg/m^3) DCM for six hours per day for four days. Accumulation of DCM in perirenal fat was found 17 hours after the last exposure. After an early uptake, the level seemed to be maintained during the four days of the experiment. A number of spontaneous behavior patterns were studied. The only ones that seemed to be affected were preening frequency and preening duration; both increased. Depression in any behavior pattern was not mentioned.

Weinstein and Diamond (1972) exposed ICR mice (17 to 25 g) continuously for three days to 10 weeks to 100 ppm (347 mg/m^3) of chemical grade DCM in a Thomas Dome with specified dynamic characteristics and at 96.66 Pa (725 mm Hg) pressure. Twelve groups of 16 mice each were used. Except for one instance at week 2, body weights were comparable to controls. Liver weights followed body weights, and in all cases liver to body weight ratios were within control limits. Triglycerides showed an approximate threefold increase at week 2, a nearly fourfold increase by week 3, then fell to about double at week four. Four mice withdrawn at three days showed no abnormalities, but at 7 days, centrilobular fat accumulation was seen accompanied by a decrease in liver glycogen. These abnormalities persisted to the termination of the experiment at 10 weeks. During this time, the nuclei enlarged. No other histopathology was seen under the light microscope. Under the electron microscope, autophagic vacuoles containing debris appeared in the hepatocytes. The smooth and rough endoplasmic reticulum showed no changes.

Haun et al. (1972) exposed mice, rats, dogs, and monkeys to 25 and 100 ppm (87 and 347 mg/m^3) reagent grade DCM for continuous exposure periods up to 100 days in Thomas Domes at ambient pressure. This is the longest continuous exposure study reported. Strain, sex and size of the test animals were not specified. Exposure to the lower concentrations had no observable effect, but exposure to the higher concentrations resulted in positive fat stains and vacuolization. The rats showed nonspecific tubular degeneration and regeneration in the kidneys, but no changes in organ-body weight ratios.

Heppel et al. (1944) conducted two inhalation experiments. One used 1,700 mg/m^3 (489 ppm) commercial DCM, for 7 hours daily, 5 days per week for six months. The other involved exposure for four hours per day, five days per week, for 7-8 weeks at a concentration of 34,000 mg/m^3 (9,780 ppm). Dogs, rabbits, guinea pigs and rats were used in each experiment. Two monkeys were added to the experiment at the higher concentration. Animals were exposed together in a single chamber. Temperature and humidity were uncontrolled. At the lower dose, 3 of 14 male guinea pigs died. They had fatty degeneration of the liver and pneumonia. No other animals' deaths attributed to DCM exposure were reported.

At the higher dose, the dog experiment was terminated after six exposures because of the continuing Stage II excitement reactions. Three rabbits and one rat died during the course of the experiment. Each animal had extensive pulmonary congestion. Clinical observations in the dogs at the lower dose showed no changes in blood pressure, blood chemistry or liver function tests, and at autopsy, organ weights of liver, kidneys, heart, lungs and spleen were similar to controls. No lesions due to DCM were found. All animals showed clinical effects after exposure to the higher concentration. Aside from the responses in dogs noted above, the other animals showed progressive signs of depression, usually becoming prostrate at the end of each daily exposure period. All animals, including dogs, recovered rapidly upon removal from the chamber, and fed well. At autopsy, dogs and guinea pigs showed fatty degeneration of the liver. Pulmonary congestion was found in the rabbits. Monkeys and rats showed no lesions related to DCM exposure.

Norpoth et al. (1974), when studying the possibility of enzyme induction in inhalation of hydrocarbon solvents, tested DCM. Male SPF Wistar rats (80-100 g) were exposed 5 hours per day to vapors containing 0, 500, or 5,000 ppm (0, 1,737 or 17,370 mg/m^3 DCM) of the hydrocarbon solvents in an exposure period of 10 days or 250 ppm (869 mg/m^3 DCM) of the solvents in an exposure period of 28 days. There were 50 animals in the control group and six animals in each of the exposed groups. At the end of these exposure periods, the animals were killed and the concentration of liver cytochrome P-450 and microsomal aminopyrine demethylase activity were determined.

Following the 10-day exposure, a significant increase in liver cytochrome P-450 in animals exposed to 500 ppm (1,737 mg/m^3), but not in animals exposed to 5,000 ppm (17,370 mg/m^3), of the compound was reported. In contrast, aminopyrine demethylase activity was not elevated in animals exposed to 500 ppm (1,737 mg/m^3) DCM but was substantially elevated in those exposed to 5,000 ppm (17,370 mg/m^3) DCM. After 28 days of exposure to DCM at 250 ppm (869 mg/m^3), no changes were noted in liver enzymes, liver weight, or histologic appearance.

These results indicate that differential enzymatic induction can be produced by exposure to DCM. The inverse dose-response relationship noted for cytochrome P-450 may be due to the combined effect of DCM and CO. Acclimatization may have occurred by the 28th day of exposure to ameliorate the effects seen at 10 days. In addition, the doses given in the second exposure period (28 days) were much smaller than during the first period.

Ten adult Wistar rats were exposed to 0 or 503 ± 85 ppm (1,747 ± 295 mg/m^3) DCM 6 hours per day for 4 days (Savolainen et al., 1977). Biochemical analysis of tissue from the right cerebral hemisphere showed no difference in protein, RNA, or glutathione levels as compared with levels in control animals. Relatively small increases in acid proteinase and nonspecific cholinesterase activities were reported, but the determinations for treated animals were made by means of only two assays and therefore may be of questionable significance.

5.4 TERATOGENIC, EMBRYOTOXIC, AND REPRODUCTIVE EFFECTS

It is not possible, on the basis of limited available data, to define the full potential of DCM to produce adverse teratogenic or reproductive effects. Human epidemiology studies have not been conducted to evaluate the effects of DCM on the exposed population. The available mammalian studies were not properly designed to evaluate the ability of DCM to produce a teratogenic response over a wide range of doses which include doses high enough to produce signs of maternal toxicity and lower doses which do not produce maternal toxicity. The teratology studies in laboratory animals were performed in rats and mice using only single doses of DCM which produced signs of maternal toxicity. Other studies in chicken embryo have indicated that DCM disrupts embryogenesis in a dose-related manner, however, since administration of DCM directly into the air space of chicken embryo is not comparable to administation of dose to animals with a placenta, it is not possible to interpret this result in relationship to the potential of DCM to cause adverse human reproductive effects. Another preliminary study in rats indicated that adverse behavioral effects may occur after exposure to low levels of DCM. However, additional behavioral studies have not been conducted to more fully evaluate this effect. In summary, although several studies have been conducted to evaluate the ability of DCM to cause adverse teratogenic, embryotoxic and reproductive effects, the limitations of the available data do not allow for a full assessment of these effects. A better assessment of these effects could be performed if the available studies met criteria similar to those suggested for teratogenicity and reproductive testing (U.S. EPA, 1978; U.S. EPA, 1979).

5.4.1 Animal Studies

The teratology studies that have been conducted on DCM are summarized below. The teratogenic and fetotoxic potential of DCM has been evaluated in the avian embryo system, and in the rat and mouse. These studies were conducted in accordance with current teratogenic testing methodology using reagent or technical grade DCM. The presentation subscribes to the basic viewpoints and definitions of the terms "teratogenic" and "fetotoxic" as summarized and stated by Chernoff (1980):

> Generally, the term "teratogenic" is defined as the tendency to produce physical and/or functional defects in offspring *in utero*. The term "fetotoxic" has traditionally been used to describe a wide variety of embryonic and/or fetal divergences from the normal which cannot be classified as gross terata (birth defects) -- or which are of unknown or doubtful significance. Types of effects which fall under the very broad category of fetotoxic effects are death, reductions in fetal weight, enlarged renal pelvis edema, and increased incidence of supernumary ribs. It should be emphasized, however, that the phenomena of terata and fetal toxicity as currently defined are not separable into precise categories. Rather, the spectrum of adverse embryonic/fetal effects is continuous, and all deviations from the normal must be considered as examples of developmental toxicity. Gross morphological terata represent but one aspect of this spectrum, and while the significance of such structural changes is more readily evaluated, such effects are not necessarily more serious than certain effects which are ordinarily classified as fetotoxic--fetal death being the most obvious example.
>
> In view of the spectrum of effects at issue, the Environmental Protection Agency (EPA) suggests that it might be useful to consider developmental toxicity in terms of three basic subcategories. The first subcategory would be embryo or fetal lethality. This is, of course, an irreversible effect and may occur with or without the occurrence of gross terata. The second subcategory would be teratogenesis and would encompass those changes (structural and/or functional) which are induced prenatally, and which are irreversible. Teratogenesis includes structural defects apparent in the fetus, functional deficits which may become apparent only after birth, and any other long-term effects (such as carcinogenicity) which are attributable to *in utero* exposure. The third category would

be embryo or fetal toxicity as comprised of those effects which are potentially reversible. This subcategory would therefore include such effects as weight reductions, reduction in the degree of skeletal ossification, and delays in organ maturation.

Two major problems with a definitional scheme of this nature must be pointed out, however. The first is that the reversibility of any phenomenon is extremely difficult to prove. An organ such as the kidney, for example, may be delayed in development and then appear to "catch up". Unless a series of specific kidney function tests are performed on the neonate, however, no conclusion may be drawn concerning permanent organ function changes. This same uncertainty as to possible long-lasting after effects from developmental deviations is true for all examples of fetotoxicity. The second problem is that the reversible nature of an embryonic/fetal effect in one species might, under a given agent, react in another species in a more serious and irreversible manner.

5.4.1.1 Chick Embryos

Elovaara et al. (1979) reported the toxicity and teratogenic potential of DCM relative to other aliphatic chlorinated hydrocarbons. The approximate LD_{50} of DCM was greater than 100 μmole/egg. Malformations were produced in embryos with doses of 5 to 100 μmole/egg injected into the air space of fertilized chicken eggs at 2, 3 and 6 days of incubation. An estimate of embryotoxicity was evaluated by determining the survival and death incidences after 14 days of incubation. Although teratogenicity resulted in chicken embryos in this study, extrapolation of these results to higher mammals is not possible at this time. This type of study may be appropriate for screening chemicals for comparative toxicity only (Johnson, 1981), and not for determining teratogenic potential (Karnofsky, 1965).

5.4.1.2 Mice

Swiss-Webster mice were exposed via inhalation to 4,350 mg/m^3 (1250 ppm) of methylene chloride for 7 hours daily during days 6 through 15 of gestation (Leong et al., 1975; Schwetz et al., 1975). This level was cited as twice the maximum excursion limit for industrial exposure. Two control groups were similarly exposed to filtered room air. Day 0 of gestation was determined when a vaginal plug was observed. Caesarean sectioning of dams was performed on day 18 of gestation.

Dams were evaluated for body weight gain and various organ weights. Maternal carboxyhemoglobin level determinations were performed on blood samples collected via orbital sinus puncture immediately following the third and tenth (last) exposure. Following Caesarean sectioning, fetuses were weighed, measured (crown-rump length), sexed and examined for external malformation. One-half of the fetuses in each litter were examined for soft-tissue malformations (free-hand sectioning) and one-half were examined, following staining, for skeletal malformations. One fetus in each litter was randomly selected and evaluated using histological techniques following serial sectioning.

In this study, maternally toxic effects of DCM exposure were observed, consisting of a significant increase in body weight, a significant increase in absolute liver weight and significant increases in carboxyhemoglobin values with return to control levels after 24 hours. On the basis of the maternal liver weight observations, a minimal toxicity may have occurred. The cause of the increased maternal weights is unknown. Since only the absolute liver weight was reported, and not the increases in liver weight per animal, it is not possible to be certain that the observed increased maternal weight gain was a result of gains in liver weight alone.

Of the twelve litters examined, a statistically significant number of litters contained fetuses that had a single extra center of ossification in the sternum. This common variation in mice is thought to reflect the degree of embryonic development. It is not known if this observation resulted from an acceleration in development or was a chance occurrence. The litters in exposed group were heavier than control fetuses (5.74 g vs 5.42 g); however this may be due to the fact that the average litter size was slightly smaller than controls (10 vs 12). The litters in this treatment group also had a lower incidence of delayed ossification of the sternebrae (17% vs 23%), split sternebrae (8% vs 18%), and ossification of the skull bones (25% vs 36%). Cleft palate and "rotated kidney" were observed in two (17%) of the DCM exposed fetuses, and not observed in any of the control litters. However, because of the low incidence of these effects, these may reflect spontaneous malformation rates.

5.4.1.3 Rats

A study using the same design as that used for the mice (Section 5.4.3.3), was performed in Sprague-Dawley rats (Leong et al., 1975; Schwetz et al., 1975). Rats were exposed by inhalation to DCM at 4,350 mg/m^3 (1250 ppm) for 7 hours daily on days 6 through 15 of gestation, with day 0 being the day spermatozoa were observed in smears of vaginal contents. Dams were Caesarean sectioned on day 21 of gestation. All other procedures were identical to those performed in the mouse study with the exception that in the rat study, food consumption was monitored.

No effect on maternal body weight or food consumption was observed. The average absolute maternal liver weight was significantly increased in comparison with the control values but there was no effect on the relative weight of the liver. Carboxyhemoglobin values in the dams were significantly increased during exposure but returned to control levels within 24 hours.

In the 19 litters evaluated, there was no effect on the average number of implantation sites per litter, litter size, number of resorptions, or fetal sex ratio and body weight. The incidence of dilated renal pelvis was significantly increased, but this observation is thought to indicate a slight but reversible delay in development similar to effects such as delays in sternal ossification. However, since this study evaluated only one dosage level, it is not possible to firmly establish the significance of this effect or its reversibility.

A study by Hardin and Manson (1980) using Long Evans rats evaluated the effect of exposure to DCM via inhalation at 15,660 mg/m^3 (4500 ppm) for 6 hours daily, 7 days per week to determine whether exposures before and during gestation were more detrimental to the developing conceptus than exposures before gestation only. Minimal maternal toxicity was observed consisting of increased liver weight, both absolute and relative, and elevated carboxyhemoglobin levels. The litters of rats exposed to DCM during gestation also had lower fetal body weights than controls. No other significant deleterious effect was observed.

Bornschein et al. (1980) reported on the behavioral teratogenic effects in the Long-Evans rats exposed to methylene chloride from the study by Hardin and Manson (1980). Ten rats were evaluated for general activity at 5, 10, 45 and 108 days of age, avoidance learning at approximately 4 months of age and activity following avoidance learning at approximately 5 months of age. Fetuses delivered by Caesarean section had lower fetal body weight than those which were naturally delivered. Treatment related effects were reported for animals in the general activity tests as early as 10 days of age (both sexes) and were still demonstrable in male rats at 150 days of age. No adverse effects were observed in growth rate, long-term food and water consumption, wheel running activity or avoidance learning.

In the Bornschein et al. (1980) study the number of rats per test group was small, usually one male and one female per litter. Therefore, this study should be regarded as preliminary and additional studies are needed to fully confirm these effects. It should also be noted that the entire field of behavioral teratology is in its early stages of development (Buelke-Sam and Kimmel, 1979), and the significance of alterations in behavioral effects to human risk assessment is not clearly defined.

5.5 MUTAGENICITY AND CARCINOGENICITY

Methylene chloride has been tested for its mutagenic potential in bacteria, yeast, and *Drosophila* and for its ability to cause chromosome damage in rat bone marrow cells. It is clearly mutagenic in bacteria, but it appears to be a weak mutagen. It has also been reported to cause reverse mutations, gene conversion, and mitotic recombination in yeast. It has not been shown to cause mutations in *Drosophila* but this negative result may be due to low exposures to the test organism. It has not been shown to cause chromosomal aberrations in rats. Based on the weight of evidence, it is concluded that methylene chloride is mutagenic in bacteria and yeast thus demonstrating the compound has intrinsic mutagenic potential. If the metabolism and pharmacokinetics of this compound in humans results in metabolic products which can interact with DNA, as is the case for bacteria and yeast, it may cause effects in humans as well. See evaluation of mutagenicity reports presented in section IV of Appendix, CAG report, for details of studies and a discussion and interpretation of the strengths and limitations of the data.

Conflicting results have been reported with regard to mammalian cell transforming potential of DCM. Price et al. (1978) reported that DCM transformed a continuous line of Fischer rat F1706 embryo cells grown in culture. Normally nontumorigenic when injected into newborn Fischer rats, F1706 embryo cells after exposure in culture to DCM, not only exhibited morphologic evidence of malignant transformation but also produced undifferentiated fibrosarcomas when injected into newborn Fischer rats. While DCM was more potent than trichloroethylene or methyl chloroform in this assay, the positive control, 3-methylcholanthrene, was more than a thousand times more potent than DCM. In contrast to this positive evidence of DCM mutagenicity, DCM has been found to be negative in a BALB/C-3T3 neoplastic transformation assay (Little, 1980). This test is designed to measure the ability of chemical agents to induce alterations in a population of mammalian cells from a pattern of controlled monolayer growth to one exhibiting foci of disoriented, piled-up growth against background monolayer. The altered cells usually produce tumors when they are injected into the appropriate hosts.

Friedlander et al. (1978) conducted an epidemiological study of Eastman Kodak Company (Rochester N.Y.) male workers exposed continuously to 104 to 4,176 mg/m^3 (30 to 1,200 ppm) DCM for up to 30 years. No increase was found in the incidence of cancer-related deaths when compared with three control groups consisting of other Kodak employees, and subjects taken from New York State and United States male mortality tables.

There are no well-designed animal bioassays available that positively support the suggestive evidence of carcinogenic potential indicated by the bacterial mutagenic test results. Theiss et al. (1977) examined DCM in a pulmonary tumor assay with A/St male mice. Groups of 20 mice were injected intraperitoneally three times per week for 24 weeks before sacrifice. The spontaneous occurrence of lung surface adenomas was increased in surviving animals at all three dose levels used (160, 400, 800 mg/kg), but not significantly except at the lowest dose with the highest animal survival rate (90 percent). The high mortality of the mice at the higher doses (60 and 75 percent) contributed to the equivocality of the study results. Long-term toxicological studies carried out by Heppel and Neal (1944), Heppel et al. (1944) and by MacEwen et al. (1972), in which a variety of animal species (dogs, rabbits, guinea pigs, and rats) were exposed to DCM by inhalation at levels of 500 to 10,000 ppm (1,737 to 34,740 mg/m^3) for periods of 14 weeks to 6 months. These studies did not reveal any incidences of tumors; however they were not carried out for a full lifetime. Further, they employed neither sufficient numbers of animals nor adequate controls for a meaningful carcinogenic evaluation.

The Dow Chemical Company (1980) has recently completed a two-year chronic toxicity and oncogenicity inhalation study of DCM in Sprague-Dawley rats and Syrian Golden hamsters. A dose-response increase in salivary gland sarcomas in the male rats become statistically significant at the highest dose (3,500 ppm, 12,159 mg/m^3). There were also increases in benign mammary tumors in female rats at all dose levels (500, 1500, 3,500 ppm; 1,737, 5,211, and 12,159 mg/m^3) and in male rats at the highest dose level (3,500 ppm; 12,159 mg/m^3). The response pattern of the salivary gland tumor is unusual, consisting of sarcomas only and appearing only in males.

The National Cancer Institute has completed the exposure phase of a two-year carcinogenicity study by gavage in rats and mice. The results of this study are not yet available for review. The National Cancer Institute also planned to begin an inhalation bioassay with DCM in November 1980. The data from these studies, with results of the Dow study, should be sufficient to evaluate the oncogenic hazard of DCM.

A final assessment of the carcinogenicity of methylene chloride will be deferred until information on the purity of the material used in the positive mutagenicity tests is obtained and until the results of the NTP gavage bioassay are evaluated, perhaps by late 1981. The decision to defer a final assessment is made because the additional information will help to clarify the unusual nature of the salivary gland response in the Dow study and to clarify the role of impurities in the positive mutagenic results in bacteria and yeast.

5.6 SUMMARY OF ADVERSE HEALTH EFFECTS AND LOWEST OBSERVED EFFECTS LEVELS

5.6.1 Animal Toxicity Studies Useful for Hazard Assessment

The preferred studies for hazard assessment are those which provide definite effect levels. Adverse affects are defined here as functional impairment and/or pathological lesions which may affect the performance of the whole organism or which reduce an organism's ability to respond to an additional challenge. Adverse effects which are not carcinogenic are assumed to be threshold phenomena. The threshold region of toxicity is estimated by evaluating four types of effect levels:

NOEL:	No-Observed-Effect Level: That dose level at which no statistically significant changes in effect(s) are observed in the exposed group as compared with its appropriate control.
NOAEL:	No-Observed-Adverse-Effect Level: That dose producing observable effects which do not in themselves represent known functional impairment, behavioral abnormality and/or pathological lesions which hinder the performance of the whole organism.
LOAEL:	Lowest-Observed-Adverse-Effect Level: The lowest dose in a study or group of studies producing functional impairment, behavioral abnormality and/or pathological lesions which hinder the performance of the whole organism.
FEL:	Frank-Effect Level: That dose which produces statistically significant change in adverse effects on exposed groups as compared with its appropriate control.

5.6.2 Inhalation Exposure

The effects of inhalation exposure to DCM in several animal species and in humans are summarized below.

Although DCM is highly volatile and has been used extensively as an industrial solvent, reports in the scientific literature of effects on humans are sparse. A few experimental human studies have been performed using single or repeated inhalation exposures to DCM. Although these studies provide precise information on exposure levels, they are limited by the small numbers of subjects and the restricted number of physiological and behavioral parameters assessed. One human epidemiological study has been published, although this study dealt only with mortality data. Accounts of acute or chronic overexposure of humans are compromised by uncertainties regarding the level and duration of DCM exposure and potential exposure to other chemicals. A series of subchronic animal experiments using continuous inhalation exposure to DCM and one chronic animal study with an intermittent exposure regimen can be used in conjunction with the limited human data to provide a framework for human risk assessment.

5.6.2.1 *Effects of Single Exposures*--Several experimental studies on the acute effects of DCM inhalation have been conducted with human subjects. For the most part, these studies focused on central nervous system function or carboxyhemoglobin levels. Exposure of volunteers to concentrations of DCM ranging from 200 to 800 ppm (695 to 2,779 mg/m^3) for 3 to 4 hours resulted in significant decreases in visual critical flicker frequency (CFF), performance on auditory vigilance tasks (AVT) (Fodor and Winneke, 1971; Winneke, 1974; Winneke and Fodor, 1976), and performance on psychomotor tasks (Winneke, 1974; Putz et al., 1976).

Exposure of subjects to approximately 1,000 ppm (3,474 mg/m^3) for 2 hours decreased the amplitude of visual evoked responses (VER), indicating CNS depression (Stewart et al., 1972b). These subjects reported being aware of a strong odor and a feeling of lightheadedness. Subjects exposed successively to 250, 500, 750, and 1,000 ppm DCM (1 ppm = 3.474 mg/m^3), 30 minutes per concentration, had more irregular reaction times than when unexposed, but heart rate, numerical ability, and short-term memory appeared to be unaffected by this exposure regimen (Gamberale et al., 1975).

DCM is metabolized to carbon monoxide (CO), which then combines with hemoglobin to form carboxyhemoglobin (COHb). Some of the acute effects of dichloromethane on the central nervous system and the heart are similar to those of CO (NIOSH, 1972) and are thought to be due to CO (NIOSH, 1976). Male nonsmokers exposed to 50 ppm DCM (174 mg/m^3) for 7.5 hours had blood COHb levels of 2.9 percent as compared to their baseline levels of 0.9 percent (Stewart et al., 1973). During exposure to 50 ppm (174 mg/m^3), COHb levels in the blood appeared to reach a plateau at about 3.2 percent towards the end of a 16-hr exposure (Fodor and Roscovanu, 1976). Smokers have been reported to have 4.6 to 5.2 percent COHb blood levels (Stewart et al., 1974; Kahn et al., 1974). Peterson (1978) reported that regardless of whether a person smoked or not, exposure to 50 or 500 ppm (174 or 1,740 mg/m^3) DCM for 7.5 hr elevated the COHb level 1.5 or 10 percent, respectively, over baseline levels. The increase in COHb levels was proportional to the concentration of DCM inhaled and the duration of exposure.

Accidental exposures of humans to high concentrations of DCM vapor have resulted in narcosis, elevated blood COHb levels (to saturations as high as 19 percent), irritation of the eyes and respiratory tract, and sometimes death (Moskowitz and Shapiro, 1952; Bonventre et al., 1977; Benzon et al., 1978; Hughes, 1954; Riley et al., 1966). One 66-year-old man suffered acute myocardial infarctions immediately after each of three separate exposures to a paint stripping compound that contained 80 percent DCM (other ingredients were not specified) (Stewart and Hake, 1976).

In none of these cases was the level of exposure measured and the duration of exposure was usually not known. Riley et al. (1966), however, measured the concentration of DCM in the breath of a worker who had been inadvertently anesthetized by a 4-hr exposure to DCM. On the basis of breath concentrations of DCM, measured at intervals after removal of the man from the exposure area, and known pharmacokinetics of DCM breath excretion, Riley et al. (1966) estimated that the man had been exposed to 8,000 to 10,000 ppm (27,792 to 34,740 mg/m^3) DCM.

Compiling the available human data, the following relationships between levels of acute inhalation exposure to DCM and effects may be tentatively suggested:

Concentration	Length of Exposure	Effects
50 ppm	7.5 hours	elevation of COHb by about 1.5% over baseline values to about 2.9% in nonsmokers
200-800 ppm	3-4 hours	impairment of perception and psychomotor performance; increased COHb levels proportional to level and duration of exposure; possible lightheadedness at high end of exposure range
1,000 ppm	2 hours	slight CNS depression; lightheadedness; awareness of strong odor; no irritation
8,000-10,000 ppm	4 hours	narcosis; life-threatening with prolonged exposure

These hypothetical dose-response relationships are rough approximations and contain several previously discussed areas of uncertainty.

Although DCM would appear to have a perceptible odor at concentrations lower than those suspected of causing narcosis, some of the workers inadvertently anesthetized by vapors of DCM had been warned to leave the worksite at once if they detected the odor of the solvent. None of the men recalled smelling DCM (Moskowitz and Shapiro, 1952).

Data from experiments with animals can be used to illuminate the effects of DCM, particularly for exposures to high concentrations. Exposures of mice, rats, and rabbits to DCM at levels of 500 to 3,000 ppm (1,737 to 10,422 mg/m^3) produced dose-related increases in total sleep time and in the intervals between REM episodes, decreases in spontaneous running activity and increases in blood COHb levels (Fodor and Winneke, 1971; Thomas et al. 1971; Berger and Fodor, 1968, cited in NIOSH, 1976; Fodor et al., 1973; Roth et al., 1973). A study with dogs indicated that repeated 2-hour exposure of the animals (through a tracheostomy) to 1,000 to 5,000 ppm (3,474 to 17,370 mg/m^3) DCM decreased the heart rate but increased blood pressure, coronary blood flow, myocardial contractility, and COHb levels, and induced cardiac arrythmias (Aviado et al. 1977). A separate study in rabbits (Taylor et al. 1976) correlates well with the dog data. Similar effects have been noted with CO (NIOSH, 1972). Flury and Zernik (1931) had reported that 4,000 or 6,000 ppm (13,896 or 20,844 mg/m^3) DCM produced light narcosis in cats, rabbits, guinea pigs, and dogs in about 1 to 6 hours.

LC_{50} values for 6- to 7-hr exposure were 14,100 to 16,100 ppm (48,983 to 55,931 mg/m^3) for rats and about 11,500 ppm (39,951 mg/m^3) for guinea pigs (Gradiski et al., 1974; Svirbely et al., 1947; Balmer et al., 1976). Rats entered a state of deep narcosis followed by complete cessation of brain electrical activity after 6 hours of exposure to 16,000 to 18,000 ppm (55.6 to 62.5 g/m^3) or 1.5 hours of exposure to 25,600 to 28,000 ppm (88.9 to 97.3 g/m^3) (Berger and Fodor, 1968, cited in NIOSH, 1976). Concentrations of 25,000 or 50,000 ppm (86.8 or 173.7 g/m^3) DCM given to anesthetized dogs and rabbits for 5 minutes or less produced primary depression of myocardial contractility

(Aviado et al., 1977; Taylor et al., 1976). Similar results were obtained with dogs exposed to a paint stripper containing 90 percent DCM (Aviado et al., 1977). Sensitization of the heart to epinephrine was produced in dogs by high concentrations of DCM; the EC_{50} for this response was 24,000 ppm (83.4 g/m^3) for a 5-minute exposure (Clark and Tinston, 1973).

The animal data indicate that DCM produced slight CNS effects at exposure levels similar to those which produced minor CNS effects in humans and also extending into a higher range than was tested on humans (up to 3,000 ppm; 10.4 g/m^3). An exposure estimated to have caused unconsciousness in a human, 8,000 to 10,000 ppm (27.8 to 34.7 g/m^3) for 4 hours, is bracketed by the production of light narcosis in several species of animals at 4,000 to 6,000 ppm (13.9 to 20.8 g/m^3) (1 to 6 hours) and the production of deep narcosis and death in rats and guinea pigs at 11,500 to 16,100 ppm (39.9 to 55.9 g/m^3) (6 to 7 hours). Although marked adverse effects on the heart (i.e., primary depression of myocardial contractility and sensitization of the heart to epinephrine) were observed in animals only at very high exposure concentrations (about 25,000 ppm; 86.9 g/m^3), some cardiovascular changes were noted in dogs exposed to 1,000 to 5,000 ppm (3,474 to 17,370 mg/m^3). Hence, acute exposures lower than those which produce narcosis may still carry an element of danger because of potential cardiovascular effects.

5.6.2.2 Effects of Intermittent or Prolonged Inhalation Exposure--Limited information is available on the effects of subchronic or chronic continuous exposures to DCM on humans or experimental animals. Most of the studies either involve occupational exposures or are designed to mimic occupational exposures. Consequently, while the data described below may be directly applicable to estimating effects from occupational exposures, an additional element of uncertainty must be considered in any attempt to estimate the effects of continuous exposures that may occur from ambient air.

As was the case for single exposures, the experimental studies of the effects on humans of repeated exposure to DCM focused on CNS function and carboxyhemoglobin levels. Results from behavioral tests of subjects exposed to 50, 100, or 250 ppm (173.7, 347.4, or 868.5 mg/m^3), 5 days a week for up to 5 weeks were inconclusive. These tests included the Romberg equilibrum test, alertness and coordination tests, and arithmetic tests (Stewart et al., 1972a,b).

Nonsmokers exposed to 50, 100, 250, or 500 ppm (173.7, 347.4, 868.5, or 1,737 mg/m^3) for 7.5 hours a day for 5 consecutive days had elevated blood carboxyhemoglobin levels. After exposure to 50 ppm (173.7 mg/m^3), COHb levels returned to baseline by the start of the next day's exposure. After exposure to 100 ppm (347 mg/m^3) or greater, COHb levels were still somewhat elevated by the start of the next day's exposure, returning to baseline over the weekend. Although there was no consistent accumulative increase in COHb levels with daily exposure (Figure 4-7) (Fodor and Roscovanu, 1976), peak values tended to be higher on the fifth day (Stewart et al., 1973). Peak COHb levels, which occurred at the end of exposure or 1 hour after the end of exposure to 50, 100, 250, or 500 ppm (173.7, 347.4, 868.5 or 1,737 mg/m^3) DCM were 2.9, 5.7, 9.9, and 11.7 percent, respectively, on the fifth day (Stewart et al., 1973). COHb levels greater than 5 percent can adversely affect patients with angina pectoris or other cardiovascular diseases (Aronow and Isbell, 1973; Anderson et al., 1973; Scharf et al., 1974). As detailed in Sections 4.3, 5.2.4, and 6.1.1, the elevated COHb levels derived from DCM metabolism are more sustained than those resulting from exposure to exogenous CO and are additive to COHb levels resulting from smoking and other exogenous CO exposure.

An epidemiological study of male Eastman Kodak workers exposed primarily to DCM gave no indication of increased risk for death from circulatory disease (including the subcategory ischemic heart disease) or other causes (Friedlander et al., 1978). The workers had been exposed to time-weighted average levels of 30 to 125 ppm (104 to 434 mg/m^3) DCM (estimated both from air monitoring and from blood COHb) for up to 30 years. Two studies were done: 1) a proportionate mortality study, using death certificates from 334 exposed workers who died from 1956 to 1976, and 2) a cohort mortality study (non-concurrent prospective) involving all 751 workers employed in the exposure area in 1964 and also separate analysis of a subgroup of 252 of these workers who had a minimum of 20 years' exposure as of 1964. This subgroup was analyzed separately to facilitate the demonstration of effects requiring long latency periods. The follow-up period in the cohort mortality study was 13 years. Comparisons were made with three different control groups: other Eastman Kodak male employees working in production activities but not exposed to DCM, New York State male age- and cause-specific mortality rates, and United States male age-specific mortality rates. Follow-up for workers aged 25 or more as of 1964 was greater than 97 percent.

Another epidemiological study (Ott et al., 1980a, 1980b; Skory, 1980; Skory et al., 1980a, 1980b), not published as of this writing but cited briefly by Burek and coworkers (1980), has also apparently failed to reveal adverse health effects attributable to DCM.

Reports of prolonged human exposure to higher concentrations of DCM are anecdotal in nature and, hence, not particularly useful for risk assessment. A 39-year-old chemist exposed occupationally for several hours a day to about 660 to 900 ppm (2,293 to 3,127 mg/m^3) DCM in the air and also dermally to liquid DCM complained of restlessness, palpitations, forgetfulness, insomnia, and a feeling of drunkenness after 3 years' exposure. He began to have auditory and visual hallucinations at the end of 5 years' exposure. He was diagnosed as having encephalosis. Another individual, exposed to 300 to 1,000 ppm (1,042 to 3,474 mg/m^3) DCM for 3 years, developed bilateral temporal lobe degeneration. This condition was attributed to chronic CO intoxication resulting from DCM exposure (Barrowcliff, 1978; Barrowcliff and Knell, 1979).

The effects of continuous exposure to DCM have been studied only in experimental animals exposed for relatively short periods of time (1 to 14 weeks). This research was undertaken because of concern about the possible exposure of astronauts to DCM emanating from construction materials in space cabins. Results, obtained with technical grade DCM and reported in a series of publications (Thomas et al., 1972; Haun et al., 1972; Weinstein et al., 1972; MacEwen et al., 1972), can be summarized as follows. Mice exposed to 25 or 100 ppm (87 or 347 mg/m^3) DCM continuously for 14 weeks had an increase in spontaneous activity at the lower concentration but not at the higher one. No gross or histological lesions were found at autopsy except that livers of the mice exposed to 100 ppm (347 mg/m^3) stained positive for fat. Hexobarbital sleep time was unaffected, but hepatic levels of cyctochromes were somewhat altered. Rats subjected to the same exposure regimens had nonspecific renal tubular degeneration and regeneration and hepatic cytoplasmic vacuolization and positive fat staining. No specific macro- or microscopic organ changes or changes in hematologic or clinical chemistry values were noted in the small number of dogs or monkeys studied. Carboxyhemoglobin levels were elevated in monkeys at both exposure levels and in dogs only at the higher exposure but there was no cumulative increase in COHb over the period of exposure. No overt signs of toxicity or changes in body weights relative to controls were noted in any of these four species.

Higher levels of continuous exposure were also investigated. Exposure of the same four species to 1,000 or 5,000 ppm (3,474 or 17,370 mg/m^3) resulted in severe signs of toxicity at the higher dose: narcosis for the first 24 hours and pronounced lethargy for the remainder of the exposure period, reduced food consumption, and high rates of mortality in mice, dogs, and monkeys. Rats were somewhat less sensitive; none died. Liver and kidney damage were commonly found in all species. At the lower exposure level, 1,000 ppm (3,474 mg/m^3), only the dogs were severely affected and died. Mice and rats did not show overt signs of toxicity but body weight gain was slightly depressed in the rats. Less severe histopathological changes than had been seen at 5,000 ppm (17,370 mg/m^3) were noted in the livers of all four species and in the kidneys of rats exposed to 1,000 ppm (3,474 mg/m^3). Monkeys had no significant changes in hematologic or clinical chemistry values.

The only chronic animal study available at this time is the 2-year inhalation study performed by Dow Chemical Company (Burek et al., 1980). Sprague-Dawley rats (SPF-derived; 192/sex/exposure concentration) and Golden Syrian hamsters (about 108/sex/exposure concentration) were exposed to 0, 500, 1,500, or 3,500 (0, 1,737, 5,211, or 12,159 mg/m^3) of DCM of greater than 99 percent purity. Exposures were for 6 hours a day, 5 days a week for up to 2 years; interim kills were performed.

With the rats, none of the exposure levels affected body weights, clinical chemistry, hematologic, or urinalysis values. Carboxyhemoglobin levels ranged from 0 to 5.3% in controls and from 8.9 to 20.4% in the treated rats, but there was no dose-response relationship and no increase with time of exposure. The lack of dose-response is probably a reflection of the saturability of DCM metabolism to CO (see Chapter 4). Beginning in the 18th month the high-dose females showed a statistically significant increase in mortality. All the rats exposed to 3,500 ppm (12,159 mg/m^3) appeared to be sluggish for about the first week of exposure.

In rats, the only organ that had definite, statistically significant, exposure-related changes was the liver. An increased incidence of hepatocellular vacuolization consistent with fatty change was noted at all exposure levels in both sexes. The severity tended to be slight; incidence and severity increased in a dose-related manner. Multinucleated hepatocytes, which according to the authors occur spontaneously in aging female rats, were found in significantly greater percentages of treated females than in controls, but incidence did not increase with increasing dose. Male rats had an increased incidence of hepatocellular necrosis starting at 1,500 ppm (5,211 mg/m^3); females had a higher incidence of coagulation necrosis at 3,500 ppm (12,159 mg/m^3). Foci and areas of altered hepatocytes, another geriatric lesion found in these female rats, were increased only in females exposed to 3,500 ppm (12,159 mg/m^3). The number of animals with neoplastic nodules or hepatocellular carcinomas was, however, not increased in any exposure group. Liver weights and liver to body weight ratios were increased for both males and females at 18 months of exposure to 3,500 ppm (12,159 mg/m^3).

This Burek et al. (1980) study would appear to establish a chronic LOEL for rats at 500 ppm (1,737 mg/m^3) for exposures occurring 6 hours/day, 5 days/week. The liver changes observed at this level were slight and no clearly deleterious effects were observed.

Hamsters were less sensitive to DCM than rats at these exposure levels. Although carboxyhemoglobin levels were higher than had been observed in the rats, no clear evidence of toxicity was found in hamsters. The effects observed in hamsters appeared to result primarily from decreased deposition of amyloid in their tissues (amyloid deposition is a naturally occurring geriatric disease in hamsters) and were not deleterious.

The data described in this section and in 5.6.1.1 concerning the effects of DCM exposure on humans and experimental animals, taken together, give some idea of the threshold region for effects of DCM exposure. Workers exposed occupationally in the range of 35 to 125 ppm (121 to 434 mg/m^3) did not have an increased risk of death from circulatory diseases or other causes (Friedlander et al., 1978). Daily 7.5-hour exposures of humans to this amount of DCM did, however, cause an increase in blood COHb to peak levels of about 5.7 percent that did not return to baseline within 24 hours (Stewart et al., 1973). Single exposures of humans to levels of DCM as low as 200 ppm (695 mg/m^3) (4 hours) have been reported to produce a depression of eye-hand coordination in demanding situations (Putz et al., 1976). Several species of animals tolerated continuous exposure to 100 ppm (347 mg/m^3) DCM for 14 weeks (Section 6.1.2) or intermittent exposure to 500 ppm (1,737 mg/m^3) (6 hours/day, 5 days a week; time-weighted average of 90 ppm for a week's exposure) for up to 2 years (Burek et al., 1980) with only minimal effects. Continuous exposure to 1,000 ppm (3,474 mg/m^3) DCM for 14 weeks produced frank toxic effects in experimental animals (supra vide) and a single 2-hour exposure to 1,000 ppm (3,474 mg/m^3) produced slight CNS depression in humans (Stewart et al., 1972). Based on the human and animal data summarized in this chapter, 200 ppm (695 mg/m^3) probably represents a low-effect level for humans, rather than a NOEL. The ACGIH has recently lowered the TLV to 100 ppm (347 mg/m^3) and NIOSH (1976) has recommended a criterion of 75 ppm (260 mg/m^3).

The study conducted by Dow Chemical Company (Burek et al., 1980) identified 500 ppm (1,737 mg/m^3) (6 hours/day, 5 days/week) as a chronic LOEL for noncarcinogenic effects in rats, the more sensitive of the two species tested. This level of exposure produced a statistically significant increase in the numbers of male and female rats with hepatocellular vacuolization characteristic of slight fatty change and a statistically significant increase in the numbers of females with multinucleate hepatocytes. Behavior, body weights, liver weights, macroscopic liver appearance, gross and histological appearance of other organs (excluding neoplastic changes), clinical chemistry (including SGPT), hematology, urinalysis, and mortality were unaffected. As described in Section 6.1.2, the incidence and severity of liver effects increased in a dose-related manner at the higher exposures (1,500 and 3,500 ppm; 5,211 and 12,159 mg/m^3) and frank toxic effects (i.e., hepatocellular and coagulation necrosis, increased mortality) were noted at 3,500 ppm.

The as yet unpublished epidemiological study (Ott et al., 1980a, 1980b; Skory, 1980; Skory et al., 1980a, 1980b) cited by Burek et al. (1980) appears, from the titles of the papers, to contain data on more sensitive biological end-points than were studied by Friedlander et al. (1978).

5.6.3 Oral Exposure

Very little information is available concerning the toxicity of DCM by the oral route. As summarized in Table 5-2, the oral LD_{50} for mice was reported to be 1987 mg/kg body weight (Zakhari, 1977) and oral LD_{50} values for rats were reported to be 2,388 mg/kg (Kimura et al., 1971) and 4,368 mg/kg (Ugazio et al., 1973). LD_{50} values can sometimes be used to calculate an approximate lethal dose for humans using the cubed root of the body weight ratios for interspecies conversions (U.S. EPA, 1980b; Friereich et al., 1966; Rall, 1969). This extrapolation assumes that larger species are more sensitive than smaller species. The LD_{50} values for DCM would suggest, however, that mice may be more sensitive than rats to this chemical; the use of this interspecies conversion factor may therefore be inappropriate.

No data from chronic oral studies are available. The NCI has completed a two-year carcinogenicity study with rats and mice in which the animals were given DCM by intubation. The histopathological phase of the study is now in progress. When the study is completed it may provide some data on the chronic oral toxicity of DCM.

A NOEL of 15 mg/kg/day has been derived from a subchronic oral study with rats (Bornmann and Loeser, 1967). Thirty male (80 g) and 30 female (75 g) Wistar rats were given 0.125 g DCM/. in their drinking water for 3 months. No difference in behavior, appearance, body weight, or survival of the treated animals were observed as compared to an equal number of control animals. No significant differences in hemotologic values, urinalysis, or plasma levels of non-esterified fatty acids were found in 8 to 10 male rats from each group. Urine from both treated and control animals sometimes tested positive for albumin. The blood glucose levels for 10 treated males were slightly elevated over levels for 10 control males but values for both groups, according to the authors, were within the normal range. The estrous cycles of the females, evaluated by vaginal smear tests, were unaffected by treatment. About 20 animals of each sex and treatment group were autopsied. Weights of the endocrine glands were not altered by treatment. The authors stated that histological examination of the internal organs revealed no pathological alterations.

The U.S. Environmental Protection Agency (1981) estimated that the rats had ingested about 15 mg DCM/kg body weight/day. This value is taken as a no-observed-effect level.

5.6.4 Dermal Exposure

Studies on the dermal toxicity of DCM are not adequate for quantitative risk assessment. Pharmacokinetic studies with human subjects indicate that absorption of liquid DCM through the skin is slow enough that toxic quantities would be unlikely to be taken into the body from direct contact of the solvent with the skin of the hands and forearms (Stewart and Dodd, 1964). Some individuals have complained of local pain from dermal contact with the liquid DCM; others have not (Stewart and Dodd, 1964; Hughes, 1954; Weiss, 1967). Chronic intermittent dermal contact with the solvent produced local irritation (Weiss, 1967). A workman who, narcotized by inhaling vapors of DCM, fell into a vat of the solvent, suffered acrocyanosis, labored breathing, severe chilblains, and repeated myocardial infarctions, and died 26 hours after this combined massive inhalation and dermal exposure (Kuzelova et al., 1975)

The only indication of toxicity solely from dermal exposure comes from an experiment with animals. Rats exposed dermally to 2 ml DCM for periods of up to 20 minutes had hemoglobin in their urine and voided decreased volumes of urine in the 3 hours following exposure (Schutz, 1958).

5.6.5 Responses of Special Concern

The potential of DCM to cause teratogenic, mutagenic, and carcinogenic effects is an important and controversial question. The limited data available concerning teratogenic and embryotoxic effects is discussed in Chapter 5 section 4. A detailed discussion and evaluation of the mutagenicity and carcinogenicity of DCM is presented in the appendix.

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APPENDIX

THE CARCINOGEN ASSESSMENT GROUP'S
CARCINOGEN ASSESSMENT
OF
METHYLENE CHLORIDE

EXTERNAL REVIEW DRAFT
May 29, 1981

THE CARCINOGEN ASSESSMENT GROUP'S
CARCINOGEN ASSESSMENT
OF
METHYLENE CHLORIDE

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This document has been reviewed and approved by the Chairman and staff of the Carcinogen Assessment Group, Office of Health and Environmental Assessment, U.S. Environmental Protection Agency. It has not been formally released by the EPA and should not at this stage be construed to represent Agency policy. It is being circulated for comment on its technical accuracy and policy implication.

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I. SUMMARY AND CONCLUSIONS

The data base is neither extensive nor adequate for assessing the carcinogenicity of methylene chloride. There is a marginally positive pulmonary adenoma response in strain A mice. Two negative animal inhalation studies were inadequate because they were not carried out for a full lifetime (Heppel et al. 1944, McEwen et al. 1972). Two long-term animal bioassay studies are currently in progress at the National Toxicology Program (NTP), a gavage test is nearing completion, and an inhalation test was recently started. The animals in the NTP gavage study were sacrificed in December 1980. The Dow Chemical Company (1980) has recently reported the results of chronic inhalation studies in rats and hamsters. The rat study showed a small but statistically significant increase in incidence of benign mammary tumors in female rats at all doses and in male rats at the highest dose, as well as sarcomas in male rats probably of the salivary gland origin. The response pattern of the salivary gland tumors is unusual, consisting of sarcomas only and appearing in males but not females. In hamsters, there was an increased incidence of lymphosarcoma in females only, which was not statistically significant after correction for survival.

Methylene chloride has been tested for mutagenicity in bacteria, yeast, Drosophila, and for its ability to cause chromosome damage in rat bone marrow cells. It is clearly mutagenic in bacteria but only weakly so. It has also been reported to cause reverse mutations, gene conversion, and mitotic recombination in yeast, but did not exert a genotoxic action in Drosophila or rat bone marrow cells. Based on the weight of evidence available at this time, it is concluded that methylene chloride is mutagenic in bacteria and yeast and thus, has the ability to cause genetic damage. If the metabolism and pharmacokinetics of this compound in humans results in metabolic products which can interact with DNA, as is the case for bacteria and yeast, it may cause effects in humans as well. Methylene chloride also showed an ability to transform cells using the rat embryo cell line F1706. This was not confirmed when a reportedly purer grade of methylene chloride was tested.

Only one occupational epidemiological study of methylene chloride has been reported and it showed no increased incidences of neoplasms at any sites which could be related to methylene chloride. However, the study was insensitive due to the short duration of the follow-up.

In conclusion, a final assessment of the carcinogenicity of methylene chloride will be deferred until information on the purity of the material used in the positive mutagenicity tests is obtained and until the results of the NTP gavage bioassay are evaluated, perhaps by late 1981. The decision to defer a final assessment is made because the additional information will help to clarify the unusual nature of the salivary gland response in the Dow study and to clarify the role of impurities in the positive mutagenic results in bacteria and yeast.

II. INTRODUCTION

Methylene chloride or methylene dichloride (dichloromethane, CH_2Cl_2) is a colorless, volatile, chlorinated hydrocarbon liquid with a penetrating ether-like odor. It is slightly soluble in water, and soluble in alcohol and ether. Its molecular weight is 84.9 and the boiling point is 40°C.

United States production of methylene chloride in 1976 exceeded 500 million pounds. An estimated 367 million pounds were released into the environment. The compound is most commonly used as a paint remover, a dispersive use with high human exposure. Methylene chloride is also used as a propellant for aerosol sprays; a blowing agent in foams; a degreasing agent; in the manufacture of photographic film, textiles, and plastics; and as a food additive extract. Most of the chemical is released into the home and factory; 95% of its uses are dispersive. The number of workers exposed is 2.5 million (National Institute for Occupational Safety and Health 1976).

Methylene chloride does not bioaccumulate and is not highly toxic to aquatic organisms. It is persistent in the environment, but due to its low boiling point, it disperses in the atmosphere.

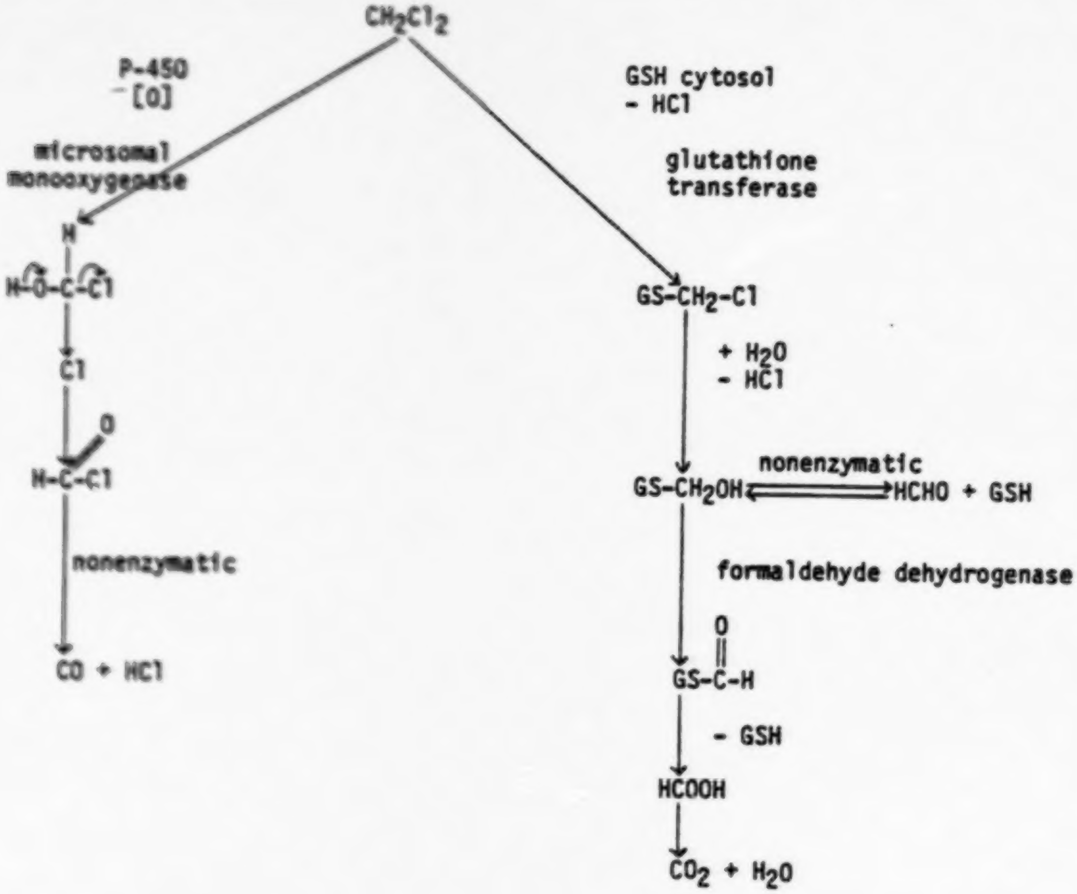
III. METABOLISM

Methylene chloride is absorbed by the lungs. Some of the absorbed chemical is metabolized but most of it is eliminated unchanged by the lungs. Radioactive methylene chloride (412 to 930 mg/kg) administered to rats intraperitoneally was eliminated via the lungs as 91% unchanged methylene chloride, 2% carbon monoxide, 3% carbon dioxide, and 1% unidentified metabolite (DiVincenzo and Hamilton 1975). Urine contained 1% of the radioactivity and the carcass 2%. Formaldehyde levels increased in serum. Rodkey and Collison (1977a) administered small doses 0.2 mM/kg (17 mg/kg) of radioactive methylene chloride to rats by inhalation or intraperitoneal injection. In this experiment a much higher fraction of metabolized methylene chloride was found compared to those discussed above; 47% of radioactivity was recovered as carbon monoxide (CO), 29% as carbon dioxide (CO_2), and none remained in the carcass. The investigator asserted that the remainder was exhaled as unchanged methylene chloride but this was not measured. A second experiment by Rodkey and Collison (1977b) showed that the conversion to CO was rate limited by enzyme saturation, and the author suggests that this fact may explain the low percent conversion observed by DiVincenzo and Hamilton (1975) who used much higher doses.

Peterson (1978) exposed 20 individuals to methylene chloride at concentrations ranging from 50 to 500 ppm for up to 7.5 hours/day for five successive days. As in animals, much of methylene chloride was excreted unchanged from the lungs. Carboxyhemoglobin levels increased in the blood of exposed individuals up to about 10% saturation. This is of concern in occupational exposure due to possible increased cardiorespiratory stress. No acceleration of methylene chloride metabolism occurred during the five exposure days. From the rate of excretion of methylene chloride after exposure stopped, it appears that much of the dose is stored in a body compartment other than the blood (probably the lipid) and released at a slower rate as compared to the rate of excretion by the lungs.

Studies of metabolism in rats have indicated two pathways for methylene chloride, one occurring in the microsomes and the other occurring in the cytosol. In the microsomes, metabolism by the mixed-function oxidases leads to carbon monoxide (Kubic and Anders 1975a, Kubic et al. 1974). An intermediate metabolite was bound to both protein and lipids (Kubic and Anders 1975b). In the cytosol, metabolism yields formaldehyde, a known mutagen and carcinogen, and requires glutathione as a cofactor (Ahmed and Anders 1976). Addition of NAD^+ decreases the formaldehyde product and causes the appearance of formic acid

(Ahmed and Anders 1978). Based on these studies the proposed pathways are:



CH2Cl2
P-450
[O]
microsomal monooxygenase
H-O-C-Cl
Cl
O
H-C-Cl
nonenzymatic
CO + HCl
GSH cytosol
- HCl
glutathione transferase
GS-CH2-Cl
+ H2O
- HCl
nonenzymatic
GS-CH2OH
HCHO + GSH
formaldehyde dehydrogenase
GS-C-H
- GSH
HCOOH
CO2 + H2O

Figure 1. Proposed metabolism of CH_2Cl_2.

The cytosol metabolism cannot be increased with pretreatment by phenobarbital or 3-methylcholanthrene as can the microsomal metabolism. It is this cytosolic glutathione-dependent metabolism that gives rise to CO_2 as an end product. The exact mechanism is not known.

Ahmed and Anders (1978) suggest that the chloromethyl glutathione intermediate would be quite reactive and may alkylate nucleophiles. Since this intermediate is an α - chloromethyl thioether, it may possess reactivities similar to bis-chloromethyl ether, a known carcinogen.

IV. MUTAGENICITY* AND CELL TRANSFORMATION

Methylene chloride has been tested for its mutagenic potential in bacteria, yeast, Drosophila, and rats. The studies presently available for review are summarized in Table 1.

There are six reports in the literature concerning the mutagenic potential of methylene chloride in bacteria. All were conducted using the Salmonella histidine reversion assay (Simmon et al. 1977, Simmon and Kauhanen 1978, Kanada and Uyeta 1978, Jongen et al. 1978, Snow et al. 1979, and Green 1980). Methylene chloride tested positive in all six studies without and/or with metabolic activation. Data were presented in five of the six reports and a clear dose-related response is apparent for each. The doses employed and the responses observed are summarized in section A of the first table. The purity and/or composition of the compound tested was not reported for any study, and the source was not given for most. The source of the material tested was given in the reports by Jongen et al. (1978) and Snow et al. (1979). Written inquiries have been made as to the purity of the tested material for all tests reviewed here (bacterial, yeast, and Drosophila) and when replies are received this information will be included in the document.

Although there is no doubt that methylene chloride is mutagenic in Salmonella, questions have been raised about the applicability of these results to predicting mutagenicity in other species, especially mammals. Green (1980) in an abstract, and in a poster format, has presented a summary of preliminary results on the comparative metabolism of methylene chloride in rat liver and in Salmonella. Although these data cannot be evaluated in detail because of their brief presentation, they are considered at some length here because of their

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*Prepared by the Reproductive Effects Assessment Group.

A. BACTERIA

TABLE 1. MUTAGENICITY TESTING OF METHYLENE CHLORIDE

Reference	Test System	Strain	Activation System	Concentration	Result	Comments
Simmon et al. 1977	*Salmonella*/S9 vapor exposure	TA 1535 TA 1537 TA 1538 TA 98 TA 100	None	(Extrapolated from figure 17) 0, 50, 100, 200, 400, and 800 ul/9 liter desiccator	(Extrapolated from Fig. 17) TA 100 Dose (ul) – Revertants/plate 0 – 170 50 – 210 100 – 300 200 – 400 400 – 650 800 – 1350	1. Toxicity not reported. 2. Number of revertants observed for TA 100 not specified numerically. 3. Data not presented for strains other than TA 100. 4. Purity and source of compound not provided.
Simmon and Kauhanen 1978	*Salmonella*/S9 vapor exposure	TA 100	Aroclor 1254 induced rat liver microsome S9 mix	0 and 1 ml/9 liter desiccator for 6.5 and 8 hours	TA 100 revertants #h – Met. Act. – Treated – Control 6.5 – - – 688 – 133 6.5 – + – 1344 – 130 8 – - – 830 – 174 8 – + – 912 – 158	1. Toxicity not reported. 2. Purity and source of compound not provided. 3. Used as a positive control in the testing of 2-chloroethyl chloroformate.

(continued on the following page)

A. BACTERIA

Table 1. (continued)

Reference	Test System	Strain	Activation System	Concentration	Result				Comments
Kanada and Uyeta 1978	Salmonella/S9 and B. subtilis rec assay testing	TA 98 TA 100	PCB induced rat liver microsome S9 mix	Not reported	Methylene chloride reported negative for both strains in B. subtilis and positive for both in S. typhimurium				1. Results summarized in abstract form. Impossible to independently evaluate. 2. Positive results of "Ames" testing supports reports by other authors using same system.
Jongen et al. 1978	Salmonella/S9 vapor exposure	TA 98 TA 100	Phenobarbital induced rat liver microsome S9 mix	(ppm x 10^3)	TA 100*		TA 98*		1. Testing conducted in gas tight perspex boxes. 2. Only highest dose exhibited less than 83% survival. 3. Purity of methylene chloride not reported.
					+S-9	-S9	+S9	-S9	
				0	152±19	129±12	21±4	19±3	
				5.7	329±37	240±32	54±5	44±6	
				11.4	515±76	407±47	74±4	56±10	
				14.1	757±62	582±56	93±9	66±12	
				22.8	865±82	653±89	123±10	96±11	
				67.0	1201±191	740±94	149±42	110±42	

*Results from three experiments, five plates/dose.

(continued on the following page)

TABLE 1. (continued)

A. BACTERIA

Reference	Test System	Strain	Activation System	Dose	Result				Comments
Snow et al. 1979	Salmonella/S9 vapor exposure	TA 98 TA 100	Methylene chloride induced Syrian Golden Hamster liver S9 microsome mix	(ul/Chamber)	TA 100*		TA 98		1. Purity of methylene not reported. 2. No information about variability of results.
					+S9	-S9	+S9	-S9	
				0	66	63	38	19	
				100	177	142	47	31	
				300	463	274	69	46	
				500	642	468	92	61	
				1000	972	632	39	72	
Green 1980	Salmonella/S9 vapor exposure	TA 1535 TA 100	Rat liver fractions	Dose (% in air)	TA 100				1. Preliminary results presented in abstract form 2. Metabolic studies conducted in rat tissue and TA 100. Similar metabolism in both systems. Radiolabel reported to bind to bacterial DNA but not to rat liver DNA.
					+S9	-S9			
				0	69±3	-			
				1.4	283±10	267±20			
				2.8	506±27	462±28			
				5.5	825±34	872±27			
				8.3	1050±88	997±88			

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*Mean calculated from three plates/dose.

(continued on the following page)

B. YEAST

TABLE 1. (continued)

					Response/10^6 Survivors				
						ade-2			
Reference	Test System	Strain	Dose (mM)	% Survival	trp-5 Conversion	Recombination	Total Genetic Alterations	ilv-1 Revertants	Comments
Simmon et al. 1977	Saccharomyces cerevisiae D3 suspension tests	Mitotic recombination							1. Data not provided, but reported negative. 2. Cytochrome P450 concentration not known. Callen et al. (1980) report different yeast strains have different levels.
Callen et al. 1980	Saccharomyces cerevisiae	D7	0	100	18	310	3300	2.7	1. Indicates ability of eukaryotic P450 dependent monoxygenase system to metabolically activate methylene chloride. 2. Active metabolites produced by this system are made intracellularly unlike exogenously employed activation systems.
			104	77	28	190	3900	4.4	
			157	42	107	4490	14000	5.8	
			209	--	--	--	--	--	

(continued on the following page)

B. YEAST

TABLE 1. (continued)

Reference	Test System	Strain	Dose (mM)	% Survival	Response/10^6 Survivors				Comments
					trp-5 Conversion	ade-2		ilv-1 Revertants	
						Recombination	Total Genetic Alteration		
Callen et al. 1980 (continued)									3. Spectral analysis of carbon tetrachloride, haloethane, and trichloroethylene conducted. Spectrum of microsomes produced by addition of trichloroethylene to suspensions of whole cells similar to that observed in mammalian microsomes. Other microsomal spectra not similar to mammalian counterparts.

(continued on the following page)

TABLE 1. (continued)

C. DROSOPHILA

Reference	Test System	Strain	Chemical	Route	No. of Chromosomes Tested	No. of Lethals	Corrected Lethals (%)	Comments
Abrahamson and Valencia 1980	*Drosophila* sex-linked recessive lethal test	FM6 females, Canton S males	EMS	Fed	773	44	5.69	1. No precaution taken to design exposure chambers to prevent evaporation of the test compound for feeding experiment. 2. No concurrent negative controls reported for the injection experiment.
			Tris	Fed	2442	35	1.43	
			Neg. Controls	Fed or Inj	94491	230	0.233	
			Methylene Chloride	Fed (1.9%)	14682	34	0.204	
				Inj (3 ul of 0.2%)	8262	18	0.157	

D. RATS

Reference	Test System	Strain	Dose (ppm)	Chromatid Break with Frag.	Chromatid Break without Frag.	Chromosome Break with Frag.	Chromosome Break without Frag.	Dicentrics	Rings	Exchanges	No. of Abnormal Cells
Johnston et al. 1980	cytogenetic evaluation of bone marrow cells Inhalation	Sprague-Dawley albino rats (Spartan substrain, SPF-derived	0	0.9 ± 0.99	0	0.2 ± 0.42	0	0	0	0	1.1 ± 1.20
			500	0.5 ± 0,71	0	0.2 ± 0.42	0	0	0	0	0.6 ± 0.69
			1500	0.5 ± 0.97	0.1 ± 0.316	0.1 ± 0.316	0	0	0	0.1 ± 0.316	0.8 ± 1.22
			3500	0.7 ± 0.48	0	0.2 ± 0.42	0	0	0.2 ± 0.42	0	1.1 ± 0.87

Comments
1. Values represent X ± SE of damage/2000 cells. 200 cells each from 10 animals/dose
2. Maximum tolerated dose may not have been approached in this study.
3. Under conditions of test, methylene chloride did not induce chromosomal aberrations.

implications with respect to the significance which should be placed on the results from bacterial tests. From previous studies, it was shown that two metabolic pathways exist for methylene chloride in rats, both involving potentially mutagenic intermediates; one involving glutathione producing the intermediate formaldehyde and the end product carbon dioxide, and the other, an oxidative pathway, is thought to form formyl chloride as an intermediate and carbon monoxide as the end product (Ahmed and Anders 1978, Kubic and Anders 1975a). In Salmonella, Green's studies using stable isotopes indicate that one or more mutagenic metabolites of methylene chloride are produced in bacteria by an oxidative pathway similar to the one existing in rat liver and that this accounts for the mutagenic activity of the compound in bacteria. Green concludes, however, that the mutagenic intermediate(s) formed by bacterial metabolism of methylene chloride is so unstable that in the Ames test it is only effective when produced inside the bacterial cell via action of bacterial metabolism and not when produced outside the cell due to metabolism by the S9 mix. The bases for this conclusion are that rat liver fractions used for metabolic activation have little effect on the mutagenicity of methylene chloride in the Ames test and in vivo radiotracer studies using ^{14}C-methylene chloride indicate that this compound or an active metabolite binds to bacterial DNA but not to rat liver DNA (Green, unpublished). By implication he indicates that the mutagenic metabolite(s) of methylene chloride is so unstable within the rat cell that it would not produce mutations. Without a more detailed description of the methods used and results observed, one is not able to critically evaluate the conclusions reached by Green, but it is important to note that experiments performed by Callen et al. (1980) in yeast described below cast doubts upon his conclusions.

Callen et al. (1980) studied the ability of methylene chloride obtained from Fisher Scientific Company (purity not reported) and six other halogenated hydrocarbons to cause gene conversion, mitotic recombination, and reverse mutations in Saccharomyces cerevisiae. They also spectrophotometrically studied cytochrome P-450 mediated metabolism of three of these compounds (carbon tetrachloride, halothane, and trichloroethylene). With respect to the mutagenicity of methylene chloride, Saccharomyces cerevisiae strain D7 log phase cells were incubated for one hour in culture medium containing 0, 104, 157, and 209 mM methylene chloride. Due to the toxicity of this compound, the genetic endpoints were not measured at the highest dose. (Percent survival for the doses utilized were 100, 77, 42, and < 0.1, respectively). The responses for the other doses (0, 104, and 157 mM) expressed per 10^6 survivors were: gene conversion at the trp-5 locus (18, 28, and 107); mitotic recombination for ade-2 (310, 190, and 4490); total genetic alterations for ade-2 (3300, 3900, and 14,000); and reverse mutations for ilv-1 (2.7, 4.4, and 5.8). A greater than twofold dose-related increase over negative controls was observed for each endpoint measured. On this basis, one concludes methylene chloride is mutagenic in yeast. No exogenously applied metabolic activation was used in these experiments and with respect to the study by Green (1980), this work by Callen et al. indicates that metabolism of methylene chloride to a mutagenic metabolite(s) results in mutagenesis in yeast as well as in bacterial cells. The positive test results obtained by Callen and coworkers using Saccharomyces for compounds which had previously tested negative in mutagenicity assays using metabolic activation (halothane, chloroform, carbon tetrachloride, and tetrachloroethylene) strengthens this conclusion and suggests that the yeast cells produce an active metabolite(s) much closer to the nucleus than is possible with extracellular liver S9 in vitro metabolic activation systems.

The second part of the study reported by Callen et al. (1980) involved an investigation of the metabolic capabilities of yeast. The metabolism of three compounds (chloroform, halothane, and trichloroethylene) was spectrophotometrically analyzed and the results indicate that P-450 dependent monooxygenases are involved in their metabolism. Although the metabolism of methylene chloride was not analyzed by Callen et al., it is reasonable to assume that P-450 dependent monooxygenases are involved in its metabolism too, since it is chemically and structurally related to the other chemicals which were analyzed and since the mutagenic response was similar for all of them.

The results of Green (1980) and Callen et al. (1980) both indicate that methylene chloride requires metabolic activation for mutagenic activity and both indicate that the mutagenic metabolic intermediate(s) is so short-lived that it must be formed within the cell to produce mutagenic activity. Callen and coworker's report of the mutagenic activity of methylene chloride in yeast raises questions about the implication of Green's work that methylene chloride would not cause mutations in mammals. Like mammals, yeast are eukaryotes and in distinction to bacteria and other prokaryotes, possess a nuclear membrane, chromatin, mitochondria, etc. The ability of the metabolite(s) of methylene chloride to produce mutations in yeast indicates its potential to produce mutations in other eukaryotes as well.

In another genetic study employing yeast, Simmon et al. (1977) reported that methylene chloride (source and purity not given, but stated to be the highest available purity) did not increase mitotic recombination in strain D3 of *Saccharomyces*. However, this may be due to strain-specific differences in cytochrome P-450 levels in D3 compared to D7, with strain D3 possessing a lower level. Differences in these levels within yeast have been reported by Callen et al. (1980) although no information was provided about strain D3. Clearly more work is needed to resolve the issues raised by Green (1980) and Callen and coworkers (1980), but at this time there is no compelling evidence to believe that the mutagenic activity of methylene chloride observed in Salmonella is unique to bacteria and not predictive of mutagenicity in other organisms.

There is one report concerning the ability of methylene chloride to mutate Drosophila melanogaster. Methylene chloride tested negative in this system. Abrahamson and Valencia (1980) conducted sex-linked recessive lethal tests using two routes of administration, feeding and injection. Due to the low solubility of methylene chloride in aqueous solutions it was not possible to utilize high concentrations of the test substance in these experiments, and the negative response observed may be due in part to the fact it was not possible to test higher doses. In the feeding study, male flies were placed in culture vials containing glass microfiber paper soaked with a saturated solution of methylene chloride in a sugar solution (1.9% methylene chloride) for 3 days. (The methylene chloride solution was added twice daily to compensate for evaporation of the compound). At this dose there was no evidence of toxicity. After mating, chromosomes from the 14,682 offspring of treated parents and chromosomes from the 12,450 offspring of concurrent control parents were assessed for recessive lethal mutations. No evidence of mutagenicity was observed. However, because of the volatility and insolubility of methylene chloride, the actual dosing to the animals may have been much less than expected.

In the injection study, 0.3 ul of an isotonic solution containing 0.2% methylene chloride was administered to male flies. This level of exposure resulted in 30% post-injection mortality. However, the post-injection mortality observed for the controls was not reported. Since the mortality observed in studies such as this is due not only to the test chemical administered, but also to the damage caused by injection, it is important to have concurrent negative controls upon which to base conclusions concerning toxicity of the test chemical administered. After mating, 8,262 chromosomes from the offspring of treated parents and 8,723 chromosomes from the offspring of control parents were assessed for recessive lethal mutations. No evidence of mutagenicity was observed by this route of administration either. Because of the low solubility of methylene chloride and its high volatility with respect to the feeding experiment, the negative results obtained after testing this chemical in Drosophila do not reduce the significance of the positive results obtained in bacteria and yeast.

There are no reports concerning the ability of methylene chloride to cause gene mutations in mammalian cells. It would be appropriate to test methylene chloride for its ability to mutate mammalian cells in culture in any adequately designed and conducted experiment.

Johnston et al. (1980) assessed the ability of a technical sample of methylene chloride (> 99% pure) to cause chromosomal aberrations in rat bone marrow cells. Doses of 0, 500, 1500, and 3500 ppm of methylene chloride were administered to the rats via inhalation 6 hours/day, 5 days/week for 6 months. Ten animals (5/each sex) comprised each exposed group. The animals were sacrificed at the end of the exposure period, bone marrow cells were collected and analyzed for cytogenetic damage. As seen in Table 1, no increased incidence in the frequency of chromosomal aberrations was observed (e.g., the $x \pm SE$ for chromatid breaks with fragments for the four groups was 0.9 ± 0.99, 0.5 ± 0.71, 0.5 ± 0.97, and 0.7 ± 0.48). This negative result may indicate that methylene chloride is not clastogenic. It may also indicate that the compound did not reach the cells of the bone marrow in sufficient concentration to cause the effects. Furthermore, it is generally recognized that chromosomal aberrations are not as sensitive an endpoint as are gene mutations (Vogel 1976). The negative results of the cytogenetic study by Johnston et al. (1980) are not considered to reduce the significance of the positive responses in bacteria and yeast.

In conclusion, methylene chloride has been tested for its mutagenic potential in bacteria, yeast, and Drosophila and for its ability to cause chromosome damage in rat bone marrow cells. It is clearly mutagenic in bacteria, but it appears to be a weak mutagen. It has also been reported to cause reverse mutations, gene conversion, and mitotic recombination in yeast. It has not been shown to cause mutations in Drosophila but this negative result may be due to low exposures to the test organism. It has not been shown to cause chromosomal aberrations in rats. Based on the weight of evidence, it is concluded that methylene chloride is mutagenic in bacteria and yeast thus demonstrating the compound has intrinsic mutagenic potential. If the metabolism and pharmacokinetics of this compound in humans results in metabolic products which can interact with DNA, as is the case for bacteria and yeast, it may cause effects in humans as well.

CELL TRANSFORMATION

Price et al. (1978) exposed Fischer rat embryo cell cultures of (F1706 subculture 108) to methylene chloride liquid at concentrations of 1.6×10^2 and 1.6×10^3 uM for 48 hours. Methylene chloride was diluted with growth medium to yield the appropriate doses. The methylene chloride sample, obtained from Fisher Scientific Company, was $\geq$ 99.9% pure. The cells were grown in Eagles minimum essential medium in Earle's salts supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 0.1 mM nonessential amino acids, 100 ug pencillin, and 100 ug streptomycin per ml. Quadruplicate cultures were treated at 50% confluency with each dose. After treatment, cells were cultured in growth medium alone at 37°C. Transformation of cells treated with either dose level of methylene chloride was observed by 23 and 30 days of incubation and was characterized by progressively growing foci composed of cells lacking contact inhibition and orientation. There was no transformation of cell grown in medium alone or in the presence of a 1:1000 acetone concentration even after a subculture. Twenty and 27 microscopic foci per three dishes with the low and high methylene chloride dose, respectively, were found in dishes inoculated with 50,000 cells from cultures treated four subcultures earlier and held for 4 weeks at 37°C in a humidified CO_2 incubator prior to staining.

Subcutaneous injection of cells treated with 1.6×10^2 uM methylene chloride five subcultures earlier produced local fibrosarcomas in 5/5 newborn Fischer 344 rats within 60 days following treatment. The ability of cells grown in growth medium alone to induce local fibrosarcomas was not determined; however, negative responses were obtained with cells grown in the presence of a 1:1000 concentration of acetone. Exposure of cells to 3.7×10^{-1} uM 3-methylcholanthrene produced 124 microscopic foci per three dishes in the inoculation test described above by 37 days of incubation and local fibrosarcoma in 12/12 rats by 27 days following subcutaneous injection of cells. The exposure of 3-methylcholanthrene was attained by initial dilution in acetone to 1 mg/ml followed by further dilution in growth medium to 0.1 ug/ml (personal communication, Dr. Price).

Dr. Price wrote a letter to the CAG dated Nov. 14, 1980 saying that "the analysis of methylene chloride showed a purity of 99.9%. The original study was done in quadruplicate and in each case the Fischer rat cells were transformed. Since the publication, the same batch of methylene chloride was sent to Andy Sivak at Arthur D. Little to be run against the Kakunago clone A31 of BALB/c 3T3. It did not transform his cells. We then repeated the study in Fischer rat cells and at the same time tested methylene chloride sent to us by the National Coffee Producers Association (NCA). The test was run in triplicate. The Fisher methylene chloride again transformed the cells, while the National Coffee Producers' (supplied by Diamond Shamrock) was negative." The different responses in the two experiments may be due to the level of impurities present in each sample. Chemical composition of different supplies are given in Table 2.

It is understood that this cell line contains the genome of the Rausher leukemia virus, but there is no basis for minimizing the positive results, since the mode of action of methylene chloride is not known i.e., due to activation of the virus.

TABLE 2. METHYLENE CHLORIDE ANALYSES[a]

	Fisher[b] Fisher D-123 Lot 761542 (ppm)	Diamond Shamrock[c] (ppm)	Dow [d] (ppm)
Methyl chloride	0.5	<1	<1
Vinyl chloride	<0.1	<1	<1
Ethyl chloride	0.8	<1	3
Vinylidine chloride	329	<1	33
Carbon tetrachloride	3.6	<1	<10
Chloroform	<0.1	26	<5
Trichloroethylene	<0.1	<1	<1
1,2-Dichlorethylene	369	20	86
Methyl bromide	---	---	11
Cyclohexane	---	300	305

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[a] Presented by Drs. Sivak and Kirshman at the Science Advisory Board Meeting, Sept. 4-5, 1980.

[b] Fisher sample used by Dr. P. Price in two series of cell transformation studies.

[c] This material is under test in NCA's chronic rat study on $MeCl_2$ in drinking water. It was also used in Dr. Price's follow-up study.

[d] This material, also analyzed by NCI, with the same results as given by Drs. Sivak and Kirshman, was used in the following tests:

NCA's 90 day studies in rats and mice
Dr. Sivak's neoplastic transformation assay
Dr. P. Price's follow-up series of cell transformation tests
NCI's chronic bioassay studies in rats and mice presently underway

V. TOXICITY

The toxicity of methylene chloride has been reviewed recently (U.S. Environmental Protection Agency 1979). One major effect is on the central nervous system producing anesthesia, sleep alterations, disturbed central nervous system function, and depression. Recovery is rapid and complete. In fatal poisonings, death is due to cardiac injury and heart failure. Methylene chloride produces cardiac arrhythmias and tachycardia. Its metabolite, carbon monoxide, increases the carboxyhemoglobin levels in the blood. Methylene chloride is an irritant to mucous membranes. Chronic exposure can cause liver damage. Methylene chloride is reportedly teratogenic to chick embryos (Elovaara et al. 1979).

VI. CARCINOGENICITY

HUMAN STUDIES

Friedlander et al. (1978)

Friedlander et al. (1978) analyzed mortality of Eastman-Kodak male employees exposed to low levels of methylene chloride. Measurements from 1959 to 1975 were in the range of 30 to 120 ppm. No increase in neoplasms, heart disease, or any other cause of death was found compared to the two control groups composed of other Kodak employees and New York State males. The population was relatively stable and the workers were rotated throughout the work area, so exposure was averaged among all the workers. Methylene chloride was used as the primary solvent in its operation for 30 years.

Two separate mortality analyses were done. One approach used the proportionate mortality ratio to examine 334 deaths of methylene chloride-exposed workers during 1956 to 1976. Seventy-one neoplasms were found; 73 were expected based on other Kodak employee mortality ratios. Furthermore, no single site was over-represented.

A second approach was a cohort mortality study of all 751 employees who were in the methylene chloride work area in 1964. Of these, 78 died during the 13-year follow-up (retrospective). Two control groups were used, other Kodak employees or New York State males, and the expected number of deaths in the exposed groups based upon these control groups was 69 and 115, respectively. The differences between the observed and the expected deaths based on the controls are not statistically significant.

Malignant neoplasms accounted for 14 of the 78 deaths in the study cohort, which was less than the 19 or 25 expected malignancies based on the control data. Five of these 14 deaths were from respiratory cancer (also less than expected based on the control groups) and four were from cancers of the digestive organs (also less than expected). Only the two deaths associated with brain or nervous tissue represented a higher than expected total (SMR = 230 and SMR = 290 vs. two control groups) but these SMR's (standard mortality ratio) were not statistically significant.

Further stratification of the cohort focused on the 252 males with 20 years or more of exposure who were employed in 1964. In this group there were 45 deaths, 7 due to malignant neoplasms (12.5 and 16.1 expected based on the two control groups) and 24 due to circulatory diseases (26 and 40.6 expected).

A further analysis of the 252 males shows that this cohort was fairly young (median age approximately 54 years in 1964). With this group the more common cancers would have to be markedly increased in order to have a reasonable probability of detecting the increase. For example, following the cohort for 13 years, cancer mortality at this age would require more than nine deaths from respiratory cancer to detect a significant result at the P = 0.05 level. This represents an increase of at least 200% over that expected, the expected probability of lung cancer death for this cohort being 0.0133 over the 13 years. For a less common cancer, liver cancer, the expected probability of death was 6.4×10^{-5}, so that two or three deaths would be statistically significant at the $P = 0.05$ level. Nevertheless, this would require a SMR = 1240. Thus, only relatively large cancer effects, at least a 100% increase would be statistically significant. Furthermore, the remaining 499 males with less than 20 years exposure were significantly younger (median age about 36 years) and a follow-up of this cohort for 13 years mortality might fail to detect even a moderate effect, since expected cancer mortality in this age group is so low.

The study appears to have been well conducted and analyzed. Further follow-up of the 20-year exposure cohort is necessary for a definitive statement.

ANIMAL STUDIES

Theiss et al. (1977)

Only one positive animal test for tumorigenicity has been reported and this was marginally positive. This involved the pulmonary tumor bioassay in mice by Thiess et al. (1977). Groups of 20 male strain A mice were injected intraperitoneally three times a week with 0, 160, 400, or 800 mg/kg for a total of 16 or 17 injections. Mice were sacrificed 24 weeks after the first injection and the lungs were examined under the dissecting microscope for surface adenomas. Some adenomas were confirmed by histology.

Tumors were found at all three dose levels; however, due to poor survival and the small number of animals, the increase in tumors did not reach statistical significance at the higher two doses (Table 3). At the lowest dose, a highly significant increase in the number of tumors was observed ($P = 0.013$).

TABLE 3. PULMONARY TUMOR BIOASSAY IN STRAIN A MICE

Dose mg/kg	Total dose given	No. mice at beginning	No. mice examined for tumors	Tumors/mouse	Significance*
0	0	20	15	0.27	---
160	2,720	20	18	0.94	< 0.013
400	6,800	20	5	0.80	> 0.1
800	12,800	20	12	0.50	> 0.1

*The test of significance used is the Exact test of ratio of two Poisson parameters.

Heppell et al. (1944)

Two inhalation studies not designed to test for carcinogenicity have been done. Heppel et al. (1944) exposed dogs, rabbits, guinea pigs, and rats by inhalation at levels of 5000 ppm for 7 hours/day and 10,000 ppm for 4 hours/day, 5 days weekly, for 6 months. No tumors developed in any animals.

McEwen et al. 1972

McEwen et al. (1972) exposed dogs to methylene chloride by inhalation at 500 ppm for 14 weeks; no tumors were reported but edema of the meninges of the brain occurred. Neither of these studies could have detected a carcinogenic response due to the short observation times.

Dow Chemical Company (1980) Inhalation Study in Rats

A total of 1,032 male and female Sprague-Dawley rats (129/sex/exposure concentration) were exposed by inhalation to 0, 500, 1500, or 3500 ppm of methylene chloride for 6 hours/day, 5 days/week (excluding holidays), in a 2-year toxicity and oncogenicity study. Approximately 95 rats/sex/exposure concentration were part of the chronic toxicity and oncogenic portion of the study and included those animals dying spontaneously, killed moribund during the study, or killed at the end of the 2-year exposure. The remaining animals were sacrificed as part of the cytogenic studies or for one of the interim kills at either 6, 12, 15, or 18 months of exposure. The rats were received at 6 to 7 weeks of age, (males weighed 220 to 250 g; females weighed 170 to 200 g) from Spartan-Research Animals, Inc., Haslett, Michigan and were individually identified using metal ear tags. All rats were maintained on a 12-hour light/dark cycle. Rats were observed daily, including weekends and holidays, for general health status and signs of possible toxicity.

Methylene chloride, representative of technical grade material, was obtained from Dow Chemical Company, Plaquemine, Louisiana, and was used throughout the exposure. Fourteen different samples of methylene chloride were analyzed during the 2 years of animal exposure; each sample showed 99% pure methylene chloride with few trace chemical contaminants that varied slightly from sample to sample as shown in Table 4. The concentration of methylene chloride vapor in chambers was considered well within the range of expected varibility. Hematologic determinations, serum clinical chemistry, urinalysis, bone marrow collection, and blood carboxyhemoglobin determination were done in animals sacrificed at 6, 12, 15, and 18 months (interim kills). Plasma estradiol determination was done at the 12- and 18-month interim kills.

TABLE 4. ANALYTICAL ANALYSIS of METHYLENE CHLORIDE
(Dow Chemical Company 1980)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Specific Gravity	1.320	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
HCl, ppm	14.2	1.8	2.3	2.9	1.8	1.1	2.5	--	--	1	--	1	1	1
H_2O, ppm	207	560	37	55	52	27	112	--	--	324[a]	--	264[a]	340[a]	33
Nonvolatile material, ppm	ND	<7	<7	<7	<7	<7	<7	ND	ND	ND	ND	ND	ND	ND
Methyl Chloride, ppm	<1	<4	<4	4.5	4.5	<4.5	4.5	1	1	1	1	1	1	1
Chloroform, ppm	60	<27	399	48	48	48	32	52	576	64	562	515	547	460
Vinylidene Chloride, ppm	60	52	53	90	75	65	60	66	72	62	78	70	77	72
Trans 1,2-Dichloro-ethylene, ppm	560	561	266	706	550	550	487	--	321	653	323	318	337	298
Cyclohexane, ppm	365	385	247	467	365	374	335	399	266	426	262	268	288	242
Ethyl Chloride, ppm	6	9	5	11	8	11	5	2	2	2	2	6	1.5	6
Vinyl Chloride, ppm	<1	--	--	--	--	--	--	1	1	1	1	1	1	1
Methyl Bromide, ppm	23	--	--	--	--	--	--	1	1	1	1	1	1	1
Carbon Tetrachloride, ppm	<2	--	--	--	--	--	--	1	15	1	13	16	20	12

ND= Not Determined

[a] Original analysis was lost; sample was subsequently reanalyzed.

--Means not detected.

This included samples from six controls/sex and four high exposure animals (3500 ppm/sex) from the 12-month kill which were pooled together (two animals/sample) to give three control samples and two high exposure (3500 ppm) samples/sex. Ten individual samples/sex (not pooled) from the high exposure and control groups were also sent from the 18-month kill.

All animals that died spontaneously, were killed in moribund condition, or were killed at the interim or terminal kills, were subjected to complete gross and microscopic pathological examinations by a veterinary pathologist. Liver samples for possible electron microscopic evaluation were collected.

In females exposed to 3500 ppm, there was a statistically significant increase of mortality from the 18th through 24th months which may be exposure-related. The remaining treated groups in males or females did not differ significantly from the controls (Table 5). There was no exposure-related difference in body weights of either male or female rats exposed to 500, 1500, and 3500 ppm of methylene chloride.

Although some hematologic values were increased and others were decreased, the mean values were within the normal range of "biological variability." Serum glutamic pyruvic transaminase (SGPT), blood urea nitrogen (BUN), and serum alkaline phosphatase (AP) values were in the normal range. It is noted that the females had significantly increased $P < 0.025$) plasma estradiol level at 18 months which may be related to the higher incidence of mammary tumors in the exposed (3500 ppm) group. Urinalysis findings were in the normal range with the exception of a few statistically significant values in specific gravity in males exposed to 1500 ppm at 6 months and male and females exposed to 3500 ppm at 12 months. Rats exposed to 500, 1500, or 3500 ppm have elevated carboxyhemoglobin values but with no evidence of either dose-response or increased values with prolonged exposure.

TABLE 5. CUMULATIVE PERCENT MORTALITY OF RATS
2-YEAR METHYLENE CHLORIDE INHALATION STUDY
(Dow Chemical Company 1980)

Month of	Males				Females			
Study	0 ppm	500 ppm	1500 ppm	3500 ppm	0 ppm	500 ppm	1500 ppm	3500 ppm
1	0.8	0	0	0	0	0	0	0
2	0.8	0	0	0.8	0	0	0	0
3	0.8	0	0	2.3	0.8	0	0.8	0
4	0.8	0.8	0	3.9	0.8	0	0.8	0
5	0.8	0.8	0	4.7	0.8	0.8	0.8	0.8
6	0.8	1.6	0.8	4.7	0.8	0.8	0.8	0.8
7	0.9	2.6	0.9	6.0	0.9	1.7	1.7	0.9
8	0.9	3.4	0.9	6.0	2.6	2.6	1.7	0.9
9	1.7	3.4	0.9	6.0	3.4	3.4	1.7	2.6
10	1.7	4.3	1.7	6.0	5.1	5.1	2.6	2.6
11	1.7	6.0	3.4	6.8	5.1	6.0	4.3	4.3
12	2.6	6.8	4.3	8.5	6.0	6.8	5.1	6.0
13	2.7	10.9	5.5	9.8	7.2	8.2	8.2	12.5
14	3.6	10.9	10.0	15.2*	9.0	8.2	8.2	12.5
15	10.9	11.8	13.6	21.4	13.5	10.9	10.0	19.6
16	15.2	16.2	20.0	29.0*	14.2	15.2	13.3	20.6
17	23.8	20.0	31.4	33.6	23.6	20.0	20.0	29.9
18	32.4	36.2	37.1	43.0	26.4	29.5	29.5	42.1*
19	44.2	45.3	49.5	54.6	36.5	42.1	46.3	57.7*
20	48.4	52.6	63.2	58.8	42.7	50.5	55.8	68.0*
21	56.8	56.8	74.7*	72.2*	49.0	58.9	65.3*	81.4*
22	63.2	65.3	83.2*	80.4*	62.5	64.2	73.7	86.6*
23	80.0	73.7	89.5	87.6	70.8	65.3	81.1	90.7*
24	85.3	85.3	93.7	92.8	78.1	74.7	86.3	95.9*

*Significantly different from control by Fisher's Exact Probability Test, $P < 0.05$.

Gross and Histologic Observations of Rats from the 6-, 12-, 15-, and 18-Month Interim Kills--

Numerous gross and histopathologic observations were recorded for control and methylene chloride-exposed rats at each time period and most were typical of spontaneous or naturally-occurring lesions normally seen in rats of this strain. There were many palpable masses in males and females. Some palpable masses appeared to be abscesses of the preputial or clitoral glands, while others were cyst-like lesions of the skin. The total number of masses in the 3500 ppm group of males were significantly increased over the controls at 15, 18, and 21 months, but not at 23 months. Female rats exposed to 500, 1500, and 3500 ppm showed an exposure-related increase in total number of masses. There was also a trend of increased benign mammary tumors in females exposed to 1500 and 3500 ppm. The total numbers of animals with benign mammary gland tumors were 9/28 (0 ppm), 10/29 (500 ppm), 11/29 (1500 ppm), and 14/27 (3500 ppm), whereas the total numbers of benign mammary gland tumors were 17/28 (0 ppm), 17/29 (500 ppm), 28/29 (1500 ppm, $P = 9.23 \times 10^{-4}$), and 37/27 (3500 ppm, $P = 2.33 \times 10^{-10}$). These observations were apparent only when the cumulative results of the 6-, 12-, 15-, and 18-month kills were evaluated.

There were a few other observations that appeared to reflect exposure-related lesions. The liver was the only organ that exhibited definite exposure-related non-neoplastic effects in both males and females at all exposure concentrations. Grossly, the effect was most prominent in females exposed to 3500 ppm and consisted of increased numbers of dark or pale foci. The control group had an incidence of 0/28, while the 3500 ppm methylene chloride-exposed female rats had a significantly greater number of foci (11/27). Some rats from the 3500 ppm exposure group had mottled livers or had an accentuated lobular pattern to the liver (0/29 control males compared to 6/27 males exposed to 3500 ppm). Because of the limited number of rats at each interim kill, this latter change may be related to exposure but may also be due to "biological variability."

Histologically observed, exposure-related lesions were present in the livers of both males and females exposed to 500, 1500, or 3500 ppm. In males, the total number of animals with any degree of vacuolization consistent with fatty changes were 5/29 (0 ppm), 19/29 (500 ppm, $P = 2.05 \times 10^{-4}$), 21/29 (1500 ppm, $P = 2.47 \times 10^{-5}$), and 23/27 (3500 ppm, $P = 2.81 \times 10^{-7}$). The liver of female rats also had alterations considered to be related to the exposure to methylene chloride. The total number of females with any degree of vacuolization consistent with fatty changes were 13/28 (0 ppm), 20/29 (500 ppm, $P = 7.16 \times 10^{-2}$), 20/29 (1500 ppm, $P = 7.16 \times 10^{-2}$), and 22/27 (3500 ppm, $P = 7.16 \times 10^{-3}$). Because of these effects, it may be considered that this experiment was performed at the maximum tolerated dose (MTD).

Gross and Histopathologic Observations of Rats Killed Moribund or Dying Spontaneously During The Study and Those From Terminal Sacrifice (24 months)--

Non-neoplastic observations--The liver was affected in both males and females exposed to 500, 1500, or 3500 ppm. The percentage of total rats with any degree of vacuolization was 17%, 38%, 45%, and 54% in the males of the 0, 500, 1500, and 3500 ppm exposure groups, respectively, and 34%, 52%, 59% and 65% in the females, respectively. Also, the degree of severity tended to increase with the dose. The males in all exposure groups had fewer cases of grossly-observed mottled and/or enlarged adrenals. These gross alterations appeared to correspond to the histologically-observed decrease in the number of cases of adrenal cortical necrosis, but the nodular hyperplasia incidence (unilateral or bilateral) was increased: 18/95 (0 ppm), 30/95 (500 ppm, $P = 3.07 \times 10^{-2}$), 31/95 (1500 ppm, $P = 2.2 \times 10^{-2}$), and 24/97 (3500 ppm).

Tumor or tumor-like lesions--The Sprague-Dawley rats used in this study normally have a high incidence of benign mammary tumors. The incidence varies slightly from study to study but normally exceeds 80% in females and about 10% in males by the end of a 2-year study. The mammary gland tumors have been classified, based on their predominant morphological cellular pattern, as fibromas, fibroadenomas, or adenomas.

The benign mammary tumor response was present in males and to a lesser extent in females. There was a non-statistically significant increase in the number of rats with a benign mammary tumor in males exposed to 3500 ppm (14/95 compared to 7/95, 3/95, and 7/95 in the 0, 500, or 1500 exposure groups). There was a slight increase in the total number of benign mammary tumors in males exposed to 0 ppm (8/95), 500 ppm (6/95), 1500 ppm (11/95), or 3500 ppm (17/97, P = 4.6×10^{-2}).

The total number of female rats with a benign mammary tumor was not increased in any exposure group (0, 500, 1500, or 3500 ppm groups had a total of 79/96, 81/95, 80/95, and 83/97 benign mammary tumors, respectively). However, the total number of benign mammary tumors increased in an exposure-related manner with 165/96 in the controls, and 218/95, 245/95, and 287/97 in the females exposed to 500, 1500, and 3500 ppm, respectively. Expressed another way, the average number of benign mammary tumors per tumor-bearing female rat increased from 1.7 in the control rats, to 2.3 in rats exposed to 500 ppm, to 2.6 in those exposed to 1500 ppm, and to 3.0 in rats exposed to 3500 ppm. This effect is exposure-related and a dose-response relationship was apparent. There was no indication of an increased number or incidences of malignant mammary tumors in either males or females.

The number of malignant tumors (Table 6) was increased in male rats exposed to 3500 ppm. This increase did not appear to clearly correlate with an

TABLE 6. SUMMARY OF TOTAL TUMOR DATA FOR RATS ADMINISTERED METHYLENE CHLORIDE FOR 2-YEARS BY INHALATION (Dow Chemical Company 1980)

		Males				Females			
	Concentration in ppm	Interim Kills	Spontaneous Deaths and Killed Moribund	Terminal Kill	Cumulative Totals	Interim Kills	Spontaneous Deaths and Killed Moribund	Terminal Kill	Cumulative Totals
Total number of rats examined	0	29	81	14	124	28	75	21	124
	500	29	81	14	124	29	71	24	124
	1500	29	89	6	124	29	82	13	124
	3500	27	90	7	124	27	93	4	124
Total number of rats with a tumor	0	9	53	13	75	14	67	21	102
	500	7	52	13	72	15	68	24	107
	1500	9	67	6	82	14	79	13	106
	3500	11	68	7	86	14	91	4	109
Total number of rats with a benign tumor	0	6	33	11	50	14	65	21	100
	500	3	29	11	43	15	60	24	99
	1500	8	42	6	56	14	73	12	99
	3500	9	37	5	51	14	84	4	102
Total number of rats with a malignant tumor	0	6	39	12	57	2	23	13	38
	500	4	45	10	59	3	28	14	45
	1500	5	48	4	57	1	31	7	39
	3500	7	55	7	69	2	32	2	36

increased number of any one tumor type or location. However, this observation led Dow Chemical Company to re-evaluate the gross and histopathologic data on all tumors arising in or around the salivary glands. Table 7 lists the specific individual animal data for these salivary gland area tumors showing the palpable mass data, specific histopathologic diagnoses, and the number of sarcomas with metastases. Table 8 summarizes the incidence of salivary gland region sarcomas in male rats.

Grossly, these tumors were large (several centimeters in diameter), cystic, necrotic, or hemorrhagic. They appeared to invade all adjacent tisues in the neck region and often completely replaced the normal salivary gland tissue. Histologically, all were sarcomas. They were composed of cells that varied from round to spindle-shaped, but appeared to be of mesenchymal cell origin. Mitotic figures were frequently observed, as were necrosis and local invasion into adjacent tissues. Most tumors had remnants of normal salivary acini or ducts that were caught-up in the cellular proliferation. Two were relatively small masses and appeared to be arising in the interstitial and capsular tissue of the salivary glands.

One tumor of this type was found in the controls (1/93) compared to 0/94 in the 500 ppm exposure group, 5/91 in males from the 1500 ppm exposure group, and 11/88 in males from the 3500 ppm exposure group ($P = 0.002$). Historically, a spontaneous incidence (0 to 2%) of this tumor type has been observed in Dow's laboratory. Therefore, the 12.5% incidence (11 of the 88 rats, Table 8) found in the males from the 3500 ppm group was higher than the corresponding controls of this study and was higher than expected based on historical control data for male rats of this strain. Also, the males exposed to 1500 ppm had five of these tumors which was also slightly higher than expected, but was not statistically significant. Therefore, this effect appeared to be exposure-related in the

TABLE 7. TIME TO TUMOR, PALPABLE MASS, AND HISTOPATHOLOGY DATA FOR SALIVARY GLAND REGION SARCOMAS IN INDIVIDUAL MALE RATS EXPOSED TO METHYLENE CHLORIDE BY INHALATION FOR 2 YEARS
(Dow Chemical Company 1980)

Animal Number	Exposure Group (ppm)	Month of Study Tumor First Observed	Size of Tumor When First Observed (cm)	Month of Study for Necropsy	Size of Tumor at Necropsy (cm)	Histological Diagnosis	Evidence of Distant Metastases
76-3301	0	15	5	15	7 x 6 x 4	Subcutaneous - Undifferentiated Sarcoma	None
76-3733	1500	16	3	17	5 x 4 x 2	Salivary Gland - Malignant Schwannoma	None
76-3809	1500	Not Observed	-	19	Not Detected (slightly enlarged salivary gland)	Salivary Gland - Malignant Schwannoma	None
76-3722	1500	24	1.5	24	4 x 3 x 2	Salivary Gland - Undifferentiated Schwannoma	None
76-3743	1500	14	2	14	No Size Given (6 grams)	Subcutaneous - Round Cell Sarcoma	None
76-3749	1500	18	3	20	5 x 4 x 4	Subcutaneous - Round Cell Sarcoma	None
76-3583	3500	Not Observed	-	24	Not Detected	Salivary Gland - Fibrosarcoma	None
76-3698	3500	14	3	15	7 x 6 x 3	Salivary Gland - Carcinosarcoma	Yes
76-3580	3500	21	2	24	9 x 6 x 4.5	Subcutaneous - Neurofibrosarcoma	Yes

(continued on the following page)

TABLE 7. (continued)

Animal Number	Exposure Group (ppm)	Month of Study Tumor First Observed	Size of Tumor When First Observed (cm)	Month of Study For Necropsy	Size of Tumor at Necropsy (cm)	Histological Diagnosis	Evidence of Distant Metastases
76-3663	3500	20	2	21	No size given	Subcutaneous - Fibrosarcoma	Yes
76-3682	3500	21	6	21	7 x 4 x 4	Subcutaneous - Undifferentiated Round Cell Sarcoma	None
76-3578	3500	16	8	16	7 x 7 x 3.5	Subcutaneous - Undifferentiated Sarcoma	None
76-3608	3500	18	2.5	18	6 x 4 x 3	Subcutaneous - Pleomorphic Sarcoma	None
76-3621	3500	17	4	18	5 x 5 x 3	Subcutaneous - Pleomorphic Sarcoma	None
76-3666	3500	15	3	16	8 x 6 x 4	Subcutaneous - Pleomorphic Sarcoma	Yes
76-3671	3500	Not Observed	-	14	3.5	Subcutaneous - Neurofibrosarcoma	None
76-3597	3500	19	6	19	7 x 7 x 3.5	Subcutaneous - ibrosarcoma	None

TABLE 8. SUMMARY OF SALIVARY GLAND REGION SARCOMA INCIDENCE IN MALE RATS IN A 2-YEAR INHALATION STUDY WITH METHYLENE CHLORIDE

Dose	Incidence	Fisher Exact Test
0 ppm	1/93 (1%)	---
500 ppm	0/94 (0%)	---
1500 ppm	5/91 (5.5%)	(P = 0.10, N.S.)
3500 ppm	11/88 (12.5%)	(P = 0.002)

males exposed to 3500 ppm.

The total number of male rats with a malignant tumor was similar in the control, 500 ppm, and 1500 ppm exposure groups. Males exposed to 3500 ppm had an increase in this category since 69 of the 124 rats had malignant tumors compared to 57, 59, and 57 in the 0, 500, and 1500 ppm exposure groups, respectively.

Dow Chemical Company (1980) Inhalation Study in Hamsters

A total of 866 Golden Syrian hamsters [Ela: Eng (syr) strain; Engle Laboratory Animals, Inc., Farmersburg, Indiana] (107 to 109/sex/exposure concentration) were exposed by inhalation to 0, 500, 1500, and 3500 ppm of methylene chloride. The materials and methods for experimental design are the same as mentioned previously in the rat portion of the Dow study. The body weights of hamsters were initially 61 to 70 grams when they were received. The hamsters were identified by a unique toe clip for group identification purposes and ear punch for individual identification within the cages.

The mortality data for males and females are presented in Table 9. Female hamsters exposed to 3500 ppm had a statistically significantly decreased mortality from the 13th through the 24th month. Females exposed to 1500 ppm also had statistically significantly decreased mortality from the 20th through the 24th month. This decreased mortality in females exposed to 3500 and 1500 ppm was considered to be exposure-related. The remaining exposure groups of male (500, 1500, and 3500 ppm) and female (500 ppm) hamsters had no differences in mortality that were exposure-related. Some hamsters of all groups had alopecia at 5 1/2 months into the study, but this alopecia was secondary to a mange mite (Demodex species) infection. This parasite did not result in an increased mortality and morbidity. No treatment-related differences were observed in the body weights of either males or females exposed to 500, 1500, or 3500 ppm of methylene chloride.

Based on the information available to the CAG, it is very difficult to conclude whether the MTD was used. Dow Chemical Company has not submitted any 90-day study (dose-finding), but a 30-day inhalation study has been reported in a letter from Dr. J. Burek to Dr. D. Singh, dated May 1, 1981. "This study was conducted prior to the 2-year study, but results have not been reported.

TABLE 9. CUMULATIVE PERCENT MORTALITY OF HAMSTERS 2-YEAR METHYLENE CHORIDE INHALATION STUDY (Dow Chemical Company 1980)

Month of Study	Males				Females			
	0 ppm	500 ppm	1500 ppm	3500 ppm	0 ppm	500 ppm	1500 ppm	3500 ppm
1	0	0	0	0	0.9	0	0.9	0
2	0	0	0	0	0.9	0	0.9	0
3	0	0	0	0	1.9	0.9	1.9	0.9
4	0	0	0	0.9	1.9	1.9	1.9	0.9
5	0	0.9	0	3.7	2.8	1.9	2.8	0.9
6	0	1.9	0	3.7	3.7	2.8	3.7	0.9
7	1.0	1.9	1.0	5.8	3.9	4.9	4.8	2.9
8	1.9	3.8	1.9	5.8	4.9	4.9	7.6	3.9
9	1.9	5.8	1.9	6.7	4.9	5.8	8.6	3.9
10	2.9	5.8	2.9	9.6	5.8	9.7	8.6	4.9
11	3.8	5.8	2.9	11.5	9.7	10.7	9.5	4.9
12	5.1[a]	6.7	3.9	12.5	13.3[a]	11.7	13.3	4.9
13	6.4	10.1	10.2	17.2*	22.6	16.3	15.0	7.1*
14	10.6	14.1	13.3	24.2	26.9	24.5	20.0	11.2*
15	14.9	17.2	14.3	26.3	32.3	26.5	24.0	15.3*
16	23.4	18.2	17.3	33.3	36.6	30.6	29.0	23.5*
17	24.5	19.2	19.4	34.3	41.9	32.7	32.0	27.6*
18	31.9	24.2	24.5	37.4	52.7	38.8	40.0	30.6*
19	36.0	29.8	31.2	42.6	63.6	59.1	50.5	40.9*
20	37.1	43.6	41.9	55.3*	71.6	63.4	55.8*	45.2*
21	41.6	51.1	54.8	60.6	80.7	70.0	61.1*	53.8*
22	56.2	61.7	68.8	69.1	88.6	82.8	68.4*	72.0*
23	61.8	63.8	75.3	74.5	94.3	91.4	75.8*	80.6*
24	82.0	78.7	88.2	85.1	100.0	95.7	89.5*	90.3*

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[a] Five males and five females died due to food deprivation. These animals were subsequently deleted from mortality calculations.

*Significantly different from controls by Fisher's Exact Probability test, $P < 0.05$.

CD-1 mice, Golden Syrian hamsters, Sprague-Dawley and CDF (F-344) rats were exposed to 0, 2500, 5000, or 8000 ppm methylene chloride vapor 6 hours/day, 5 days/week, for a total of 20, 19, 20 or 6-2/3 exposures, respectively, in 21-29 days. Body weight data was obtained throughout the study. Clinical chemistry parameters were measured. All animals underwent gross pathological examination at the termination of the experiment. The weights of the liver and kidneys were recorded from animals in the 0, 2500, and 5000 ppm groups, and organ/body weight ratios were calculated. Animals exposed to 8000 ppm methylene chloride exhibited anesthetic effects, increased blood urea nitrogen levels in Sprague-Dawley male rats, and decreased body weights in rats. The animals exposed to 5000 ppm showed slight anesthesia, decreased body weight in male rats, increased SGPT values in female mice and Sprague-Dawley rats and increased liver weights in female mice, hamsters and rats. Animals exposed to 2500 ppm methylene chloride appeared to scratch more than controls and therefore appeared to be affected, but showed no other effect attributable to exposure. The target organ in this study was the liver. Because of the results obtained, 8000 and 5000 ppm methylene chloride were considered to be too high a dose level for the two-year study, and 2500 ppm did not appear to have produced a severe enough response over the 30-day period. Therefore, concentrations of 3500 of methylene chloride was chosen as the top dose for the two-year study."

It is interesting to note that Dow conducted only a 30-day subchronic (dose-finding) study rather than the usual 90-day study which is used in most animal bioassays. Further, it should be noted that there is no decrease in body weight or mortality rate in the experimental group of hamsters as compared to the controls. Hematologic determinations, serum clinical chemistry, urinalysis, bone marrow collection, blood carboxyhemoglobin determination were done in animals at the 6-, 12-, 18-, and 24-month interim kills. No treatment-related effects were observed in any of the parameters evaluated in male or female hamsters after 6, 12, 18, or 24 months of exposure to 500, 1500, or 3500 ppm of methylene chloride, respectively.

Carboxyhemoglobin determinations were performed on the blood of male and female hamsters following 22 months on test. Males and females exposed to 500, 1500, or 3500 ppm all had significantly elevated carboxyhemoglobin values. There was a slight trend in a dose-response relationship in females since the mean carboxyhemoglobin values for those exposed to 500 ppm was 23.6% while those for females exposed to 1500 or 3500 ppm were 30.2% and 34.6%, respectively. However, an apparent dose-response relationship was not observed in males. Dow Chemical Company indicated that these data, when compared to those in the rat study, suggested that hamsters had a greater degree of metabolism of methylene chloride to carbon monoxide. Furthermore, the apparent dose-response in females was surprising. As a result, additional hamsters were exposed to a single 6-hour exposure and their carboxyhemoglobin values were determined. There was no apparent sex difference and the dose-response relationship observed in females after 22 months could not be verified. Since there was no dose-response relationship in male and female rats or male hamsters and since the female hamsters exposed to a single 6-hour exposure did not show a dose-response relationship, the apparent trend for an exposure-related increase in female hamsters at 22 months may be a cumulative effect but is probably not an exposure-related effect.

Gross and Histopathologic Observations of Hamsters from the 6-, 12-, and 18-Month Interim Kills--

A variety of gross and histopathologic observations were recorded for hamsters that were sacrificed at the 6-, 12-, or 18-month interim kills. Histopathologically, exposure-related differences were present consisting of decreased numbers of hamsters with amyloidosis of the liver, kidney, adrenals, thyroid, and spleen. There were a few animals in each group which may or may not represent the trend of amyloidosis in males.

Gross and Histopathologic Observation of Hamsters Killed Moribund or Dying Spontaneously During the Study and Those From Terminal Sacrifice (24-months)--

Neoplastic and non-neoplastic observations--A gross and histopathologic examination was conducted on all hamsters that died or were killed moribund during the study, and on all surviving hamsters at the end of the study. The histopathologic observations for males and females are presented in the Dow Chemical report (1980), tables 124 to 127. The observations shown include all the neoplastic and non-neoplastic lesions recorded for these hamsters. Most observations were within the normal or expected range for Golden Syrian hamsters as indicated by Dow Chemical Company. The female rats had increased incidences of lymphosarcoma in the experimental group. The incidence was 1/96, 6/95, 3/95, and 7/97 ($P = 0.033$) in the 0, 500, 1500, and 3500 ppm groups, respectively (letter from Hugh Farber, Dow Chemical Company, to EPA, dated April 14, 1981). A re-evaluation of lymphosarcoma data of female hamsters by the CAG resulted in the following incidences: 1/91, 6/92, 3/91, and 7/91 ($P = 0.032$) in the 0, 500, 1500, and 3500 ppm groups, respectively. The differences between the denominators above reflects the CAG's use of actual numbers of animals examined (which did not include animals that were severely cannibalized, autolyzed, or missing), whereas, the Dow denominator's included the total number of animals. It should also be noted from this table that only a small number of mammary gland tissues were examined and no lesions were found. Table 10 summarizes the total tumor data. Total number of hamsters with benign tumors was significantly increased in females at 3500 ppm and malignant tumors in males at 1500 ppm.

Discussion and Conclusions of the Dow Chemical Company (1980) Inhalation Study

Based on all the data evaluated, the following points are considered to be major findings in the rat and hamster studies:

1) Male rats exposed to 1500 or 3500 ppm appeared to have an increased number of sarcomas in the ventral midcervical area near the salivary glands. There were 1/93, 0/94, 5/91, and 11/88 ($P = 0.002$) sarcomas in male rats exposed to 0, 500, 1500, or 3500 ppm, respectively. Based on routine sections, special stains, and ultrastructural evaluations, these tumors appeared to be of mesenchymal cell origin; however, a myoepithelial cell origin of these cells could not be ruled out. These tumors had some areas that morphologically resembled one cell type (i.e., neurofibrosarcoma, fibrosarcoma), and still other tumors had cell types that were undifferentiated or pleomorphic. In some, one cell type was predominant, while in others, areas of all of the above cell types were present depending on the area of the tumor examined. Furthermore, the origin of each of these tumors remains questionable. All appeared to be arising in the midcervical region and all involved the salivary glands. Only two tumors were small enough to be localized within the salivary gland. The rest were larger tumors that clearly involved the salivary glands as well as adjacent tissues and could have been growing either into or out of the salivary glands. However, they probably arose within the salivary glands based on the two localized small tumors described above.

TABLE 10. SUMMARY OF TOTAL TUMOR DATA FOR HAMSTERS ADMINISTERED METHYLENE CHLORIDE BY INHALATION FOR 2 YEARS
(Dow Chemical Company 1980)

		Males				Females			
	Concentration (ppm)	Interim Kills	Spontaneous Deaths and Killed Moribund	Terminal Kill	Cumulative Totals	Interim Kills	Spontaneous Deaths and Killed Moribund	Terminal Kill	Cumulative Totals
Total number of hamsters during this period[a]	0	15	76	16	107	15	91	0	106
	500	10	74	20	104	10	88	4	102
	1500	10	82	11	103	10	81	10	101
	3500	15	78	14	107	14	82	9	105
Total number of hamsters with a tumor	0	4	18	3	25	2	17	-	19
	500	3	17	9	29	0	20	1	21
	1500	2	17	8	27	2	13	4	19
	3500	4	18	7	29	2	27	3	32*
Total number of hamsters with a benign tumor	0	4	14	2	20	2	11	-	13
	500	2	10	7	19	0	8	1	9
	1500	2	6	5	13	1	9	3	13
	3500	3	10	6	19	1	22	3	26*
Total number of hamsters with a malignant tumor	0	0	6	1	7	0	8	-	8
	500	1	8	4	13	0	13	0	13
	1500	0	12	3	15*	1	4	2	7
	3500	1	8	1	10	1	9	0	10

*Significantly different from controls when analyzed by Fisher's Exact Probability Test, $P < 0.05$.

-Indicates none examined or not applicable.

[a] Does not include hamsters that escaped from their cages, or hamsters that were severely autolyzed, or severely cannibalized. Also, this total does not include 500 ppm or 1500 ppm male and female hamsters from the 6-month interim kill because no histopathology was done on these animals except for a liver special stain (Gomori's Prussian Blue Iron Reaction).

Therefore, there was an apparent association between the increased incidence of sarcomas in the salivary gland region of male rats and prolonged exposure via inhalation to 1500 or 3500 ppm methylene chloride. It is interesting to note that there were no salivary gland sarcomas in female rats or in hamsters of either sex. Further, it will be of interest to find out what kind of lesions are present or absent in the National Toxicology Program study.

2) Male and female rats exposed to methylene chloride had increased numbers of benign mammary tumors compared to control values. Female rats exposed to 500, 1500, or 3500 ppm of methylene chloride had increased numbers of benign mammary tumors per tumor-bearing rat compared to the controls. The increase was evident in the palpable mass data and the gross necropsy findings which were confirmed by the histopathologic examination. The total number of female rats with a benign mammary tumor was not statistically increased in any exposure group (0, 500, 1500, or 3500 ppm groups had a total of 79, 81, 80, and 83 animals with benign mammary tumors, respectively). It should be noted that Sprague-Dawley rats have very high incidence of spontaneous mammary tumors. However, the total number of benign mammary tumors have increased in an exposure-related manner with 165/92 in the controls and 218/90, 245/92, and 287/95 in the females exposed to 500, 1500, or 3500 ppm, respectively. Expressed another way, the average number of benign mammary tumors per female rat increased from 1.7 in the controls, to 2.3 in those exposed to 500 ppm, to 2.6 in those exposed to 1500 ppm, and to 3.0 in those exposed to 3500 ppm. This increase was considered to be exposure-related and dose-dependent.

A mammary tumor response was present in male rats also, but to a lesser extent than in females. There was an increase (not statistically significant) in the number of rats with a benign mammary tumor in males exposed to 3500 ppm. There was a slight increase in the total number of benign mammary tumors in males exposed to 1500 or 3500 ppm. As was the case in females, these effects in males exposed to 1500 or 3500 ppm were considered to be exposure-related.

There were no mammary gland tumors in male or female hamsters. It is also interesting to note that only 28/92, 44/93, 30/94, and 27/93 mammary glands tissues were examined in the 0, 500, 1500, and 3500 ppm groups, respectively. Not a single lesion was recognized in the mammary gland tissues examined. The CAG feels that a greater number of hamster mammary gland tissues should have been examined to better evaluate the true incidence of mammary tumors.

3) There was an increased incidence of lymphosarcoma in female hamsters. The incidence was 1/96, 6/95, 3/95, and 7/97 (P = 0.033) in the 0, 500, 1500, and 3500 ppm groups, respectively (letter from Hugh Farber, Dow Chemical Company, to EPA, dated April 14, 1981). A re-evaluation of lymphosarcoma data of female hamsters by the CAG resulted in the following incidences: 1/91, 6/92, 3/91, and 7/91 (P = 0.032) in the 0, 500, 1500, and 3500 ppm groups, respectively. The differences between the denominators above reflects the CAG's use of actual numbers of animals examined (which did not include animals that were severely cannibalized, autolyzed, or missing), whereas, the Dow denominator's included the total number of animals. Dow Chemical Company believed that the females exposed to 3500 ppm had better survival (statistically significant) than the controls and thereby had a greater chance to develop these tumors. After correction for survival (1/39 vs. 7/63) by the CAG this data is not statistically significant (P = 0.12).

4) There appears to be a question as to whether or not the doses given the rat and hamster were at or near the MTD. The body weights of male rats

increased particularly toward the latter part of the experiment, whereas the body weight of female rats were unaffected in any experimental group. Exposure to 3500 ppm resulted in an increased mortality rate in female rats during the last six months, but the male rats were unaffected at any concentration. On the other hand, decreased mortality was observed in female hamsters exposed to 1500 and 3500 ppm while mortality in male hamsters was unaffected at 500, 1500, and 3500 ppm based on only a 30-day rat and hamster inhalation (dose-finding) study (see details, pages 39 and 41). Based on this information, it is difficult to judge whether the animals were given a dose equal to the MTD.

Other Animal Studies in Progress

Another chronic study in rats and mice started in September 1979 at Hazleton Laboratories of America, Inc. sponsored by the National Coffee Association. Methylene chloride in this experiment is being used in drinking water. Details of this study are not available. The CAG will review this experiment when the reports are available.

A bioassay for carcinogenicity has been undertaken by the National Cancer Institute in a 2-year chronic gavage study with rats and mice. The sacrifice date for this study was December 1980. The NTP has also scheduled an inhalation test in rats and mice to start in April 1981.

VII. UNIT RISK ESTIMATE

Insufficient data exist on which to base a unit risk assessment.

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